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UTILITY PATENT APPLICATION TRANSMITTAL (Only for new nonprovisional applications under 37 CFR 1	Attny Docket No. PF140P1D1	Total Pages: 103+postcard
	First Named Inventor or Application Identifier: He, et al.	
	Date: July 10, 2000	

APPLICATION ELEMENTS
See MPEP chapter 600 concerning utility patent application contents.

Assistant Commissioner for Patents
Address to: Box Patent Application
Washington, D.C. 20231

1. ☒ Fee Transmittal Form (in duplicate) [2]
2. ☒ Specification [total pgs. 54]
(preferred arrangement set forth below)
- Descriptive title of the Invention
- Cross References to Related Applications
- Statement Regarding Fed Sponsored R&D
- Reference to Microfiche Appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the Drawings (if filed)
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

3. ☒ Drawing(s) **Figs. 1-3** Total Sheets **3**
4. ☒ Oath or Declaration Total pages
a. ☐ Newly executed (original or copy)
b. ☒ Copy from a prior application (1.63(d)) (for con/div with Box 17 completed)
i. ☐ Deletion of Inventors (signed statement attached deleting inventor(s) named in prior app.)
5. ☒ Incorporation by Reference (with Box 4b)
The entire disclosure of the prior application, from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference therein.

6. ☐ Microfiche Computer Program (appendix)
7. Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)
a. ☐ Computer Readable Copy
b. ☒ Paper Copy (identical to computer copy)
c. Statement verifying identity of above copies

Accompanying Application Parts
8. ☐ Assignment Papers (cover and document(s))
9. ☐ 37 CFR 3.73(b) Statement ☐ Power of Attny
10. ☐ English Translation Document
11. ☐ Information Disclosure Statement/PTO-1449
☐ Copies of IDS Citations
12. ☒ Preliminary Amendment, with Statement Under 37 CFR §1.821(e) & (f)
13. ☒ Return Receipt Postcard (MPEP 503) (should be specifically itemized)
14. ☐ Small Entity Statement(s)
☐ Statement filed in prior application, status still proper and desired
15. ☐ Certified copy of priority document(s)
16. ☒ Other: 7 pages of formal drawings (Figs. 1A-B, 2A-B, 3A-C); Substitute sequence listing (8 pages); Power of Attorney by Assignee of Entire Interest-Revocation of Prior Power of Attorney; Declaration Under 37 CFR §1.132 with Exhibits A-E

17. If a CONTINUING APPLICATION, check appropriate box and supply the requisite information:

☐ Continuation ☒ Divisional of prior application No. 08/462,969, filed on June 5, 1995, which is a continuation-in-part of Appl. Serial No. 08/334,251, filed on November 1, 1994
☐ Continuation-in-Part (CIP)

18. CORRESPONDENCE ADDRESS

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JC760 U.S. PTO 07/10/00

jc866 U.S. PTO 09/613508 07/10/00

Via Hand Delivery July 10, 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: He, et al.

Application Serial No.: Not yet assigned — Art Unit: Not yet assigned

Filed: Concurrently herewith — Examiner: Not yet assigned

For: Interleukin-1 ß Converting Enzyme Like Apoptosis Protease-3 and 4 — Attorney Docket No.: PF140P1D1

PRELIMINARY AMENDMENT
WITH STATEMENT UNDER 37 C.F.R. §§ 1.821(e) and (f)

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination, Applicants hereby request that the following amendments and remarks be entered into the subject application.

In the Specification:

On page 1, underneath the Title "INTERLEUKIN-1 ß CONVERTING ENZYME LIKE APOPTOSIS PROTEASE-3 AND 4", please insert the following:

--CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a divisional of Application No. 08/462,969, filed June 5, 1995, which is a continuation-in-part of Application Serial No. 08/334,251, filed November 1, 1994, each of which is hereby incorporated by reference in its entirety.--

On page 4, line 20, please insert before the paragraph beginning with the phrase "The following drawings . . .", the heading:

--**Brief Description of the Drawings**--.

On page 4, line 23, delete "Figure 1" and replace therewith --Figures 1A-B--, and delete "cDNA" and replace therewith --cDNA sequence (SEQ ID NO:1)--.

On page 4, line 24, delete "sequence" and replace therewith --sequence (SEQ ID NO:2)--.

On page 4, line 28, delete "Figure 2" and replace therewith --Figures 2A-B--, and delete "cDNA" and replace therewith --cDNA sequence (SEQ ID NO:1)--.

On Page 4, line 29, delete "sequence" and replace therewith --sequence (SEQ ID NO:2)--.

On page 4, line 32, delete "Figure 3" and replace therewith --Figures 3A-C--.

On page 5, line 4, delete Figures 1 and 2" and replace therewith --Figures 1A-B and 2A-B--.

On page 5, line 11, insert before the paragraph beginning with the phrase "The polynucleotide encoding ICE-LAP-3" the following:

--These deposits are biological deposits with the American Type Culture Collection ("ATCC") located at 10801 University Boulevard, Manassas, Virginia 20110-2209. Since the deposits referred to are being maintained under the terms of the Budapest Treaty, they will be made available to a patent office signatory to the Budapest Treaty.--

On page 5, line 20, delete "hoholog" and replace therewith --homolog--.

On page 5, line 24, delete "259-263" and replace therewith --184-188--.

On page 6, line 5, delete "Figures 1 and 2" and replace therewith --Figures 1A-B and 2A-B--.

On page 6, line 10, delete "Figures 1 and 2" and replace therewith --Figures 1A-B and 2A-B--.

On page 6, line 13, delete "Figures 1 and 2" and replace therewith --Figures 1A-B and 2A-B--.

On page 6, line 29, delete "Figures 1 and 2" and replace therewith --Figures 1A-B and 2A-B--.

On page 6, line 35, delete "Figures 1" and replace therewith --Figures 1A-B--.

On page 7, line 1, delete "2" and replace therewith --2A-B--.

On page 7, line 5, delete "1 and 2" and replace therewith --1A-B and 2A-B--.

On page 7, line 11, delete "Figures 1 and 2" and replace therewith --Figures 1A-B and 2A-B--.

On page 8, line 31, delete "Figures 1" and replace therewith --Figures 1A-B--.

On page 8, line 32, delete "2" and replace therewith --2A-B--.

On page 9, line 29, delete "Figures 1 and 2" and replace therewith --Figures 1A-B and 2A-B--.

On page 10, line 8, delete "Figures 1 and 2" and replace therewith --Figures 1A-B and 2A-B--.

On page 38, line 4, delete "Example 5" and replace therewith --Example 7--.

In the Sequence Listing:

Please delete the Sequence Listing at pages 40-49 of the specification and replace it with the Substitute Sequence Listing submitted herewith. Additionally, please renumber the claims accordingly.

In the Drawings:

Please replace the originally filed Figures 1-3 with the Formal Drawings of Figures 1A-B, 2A-B and 3A-C submitted concurrently herewith.

REMARKS

The specification has been amended to claim benefit of prior filed applications in accordance with 37 C.F.R. § 1.78(a)(2), to conform to current Patent Office practice requiring reference to the lettered subparts of the formal drawings submitted herewith, to correct typographical errors, to update the address of the American Type Culture Collection, to incorporate sequence identifiers pursuant to 37 C.F.R. § 1.821(d), to incorporate the Substitute Sequence Listing submitted herewith pursuant to 37 C.F.R. § 1.821(c) and renumber the pages of the claims accordingly. Additionally, the specification has been amended on page 5 to correct the location of the pentapeptide QACRG in SEQ ID NO:2. No new matter has been added.

A substitute sequence listing and substitute Figures 1A-B and 3A-C are submitted herewith to correct nucleotide and amino acid sequence errors as originally presented in the ICE-LAP 3 sequences (shown in the sequence listing as SEQ ID NOS:1 and 2). These changes do not introduce new matter because the correct sequence was inherent to the originally sequenced plasmid clone in actual possession of the Applicants before the time the captioned application was filed.

The captioned application is directed to both polynucleotides and polypeptides which are chemical compounds. The nucleotide sequence is but an inherent property of the described polynucleotides. There is a line of chemical case law where applicants have been permitted to amend the specification to correct the formula of a chemical compound after an application's filing date, provided that it had been demonstrated that one of skill in the art would have appreciated that the applicant was in possession of the compound itself at the time of filing. The rationale is that the formula is an inherent property of the compound and thus amending the specification to correct the formula is not new matter. See *In re Nathan*, 140 U.S.P.Q. 601, 604 (C.C.P.A. 1964). *Accord Kennecott Corp. v. Kyocera Int'l, Inc.*, 5 U.S.P.Q.2d 1194, 1198 (Fed. Cir. 1987), *cert. denied*, 486 U.S. 1008 (1988) ("The disclosure in a subsequent patent application of an inherent property of a product does not deprive that product of the benefit of the earlier filing date.").

In the field of biotechnology, applicants often rely on a deposited clone, where the deposit was made prior to filing, to establish possession of nucleic acids or proteins. The focus for determining whether applicants were in possession of claimed nucleic acids or proteins has been determined, at least in part, by considering whether the applicant has: (1) established that one skilled in the art in possession of the deposited clone would have been aware of both the DNA sequence and the encoded amino acid sequence, or would be able to determine these sequences without undue experimentation, (2) established that the DNA and amino acid sequences are described in a manner such that one skilled in the art could distinguish them from other sequences, and (3) resequenced a clone which is identical to that of the deposit and established a "chain of custody" for this clone. See

e.g., *Ex parte Maizel*, 27 U.S.P.Q.2d 1662, 1669-1670 (B.P.A.I. 1992).

Submitted herewith is a Declaration of Craig Rosen Under 37 CFR §1.132 (the "Rosen Declaration") which was filed in connection with the prosecution of parent application Serial Number 08/462,969, and which describes certain events involving the characterization of the nucleotide sequence of cDNA clone HE2CA82 which encodes ICE-LAP 3. The HE2CA82 cDNA clone was deposited with the American Type Culture Collection (ATCC) on August 25, 1994; i.e., prior to the filing date of parent application US Serial No. 08/334,251 (filed November 1, 1994). Exhibit A of the Rosen Declaration is a copy of the contract for ATCC Deposit No. 75875. The Examiner will note that the present specification states on page 5, lines 7 and 8, that ATCC Deposit No. 75875 contains the cDNA which encodes ICE-LAP 3.

The corrected nucleic acid and deduced amino acid sequences (shown in replacement Figures 1 and 3 submitted herewith) were determined by reanalyzing cDNA clone HE2CA82 (ATCC Accession No. 75875); i.e., the same cDNA clone from which the originally presented sequences were determined (see paragraph 4 of the Rosen Declaration). The corrected sequence information was published by the present inventors in a peer-reviewed scientific journal article (see Exhibit B of the Rosen Declaration).

The analysis needed to determine the complete and correct sequences of cDNA clone HE2CA82 were well within the skill of the ordinary artisan as of the filing date of the parent application US Serial No. 08/334,251 (filed November 1, 1994), and such analysis would not have required undue experimentation (see paragraph 5 of the Rosen Declaration).

Exhibit D of the Rosen Declaration shows the four (4) nucleotide differences between the original sequence ("PF140" in the Exhibit) and the corrected sequence ("Duan" in the Exhibit).

In summary, because Applicants have demonstrated that the corrected sequences are inherently present in the deposited material, and because Applicants have demonstrated "chain of custody" for the material originally sequenced and the resequenced material, correction of the originally presented sequence information herein is not new matter.

Statement Under 37 C.F.R. §§ 1.821(e) and (f)

The above-identified patent application is a divisional of application Serial No. 08/462,969, filed June 5, 1995. The content of the paper copy of the Substitute Sequence Listing filed herewith is identical to the sequence content of the computer readable sequence listing previously filed on March 22, 1999 in connection with application Serial No. 08/462,969.

In accordance with 37 C.F.R. § 1.821(e), please use the computer readable form filed on March 22, 1999 in connection with application Serial No. 08/462,969 as the

computer readable form for the instant application. It is understood that the Patent and Trademark Office will make the necessary change in application number and filing date for the computer readable form that will be used for the instant application. A paper copy of the Substitute Sequence Listing is being filed herewith. Applicants hereby certify that the paper copy of the Substitute Sequence Listing filed herewith and the computer readable sequence listing previously filed in connection with application Serial No. 08/462,969 on March 22, 1999 are the same and do not include new matter.

Applicants respectfully request that the amendments and remarks above be entered and made of record in the file history of the instant application.

Respectfully submitted,

Dated: July 10, 2000

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Enclosure

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INTERLEUKIN-1 β CONVERTING ENZYME LIKE APOPTOSIS PROTEASE-3 and 4

This invention relates to newly identified polynucleotides, polypeptides encoded by such polynucleotides, the use of such polynucleotides and polypeptides, as well as the production of such polynucleotides and polypeptides. More particularly, the polypeptides of the present invention are interleukin-1 β converting enzyme like apoptosis protease-3 and interleukin-1 β converting enzyme like apoptosis protease-4, sometimes hereinafter referred to collectively as "ICE-LAP-3 and 4". The invention also relates to inhibiting the action of such polypeptides.

It has recently been discovered that an interleukin-1β converting enzyme (ICE) is responsible for cleaving pro-IL-1β into mature and active IL-1β and is also responsible for programmed cell death (or apoptosis), which is a process through which organisms get rid of unwanted cells. The present invention is directed to ICE-LAP-3 and 4 which are structurally related to ICE.

In the nematode *caenorhabditis elegans*, a genetic pathway of programmed cell death has been identified (Ellis, R.E., et al. Annu. Rev. Cell Biol., 7:663-698 (1991)). Two genes, *ced-3* and *ced-4*, are essential for cells to undergo

programmed cell death in *C. elegans* (Ellis, H.M., and Horvitz, H.R., Cell, 44:817-829 (1986)). Recessive mutations that eliminate the function of these two genes prevent normal programmed cell death during the development of *C. elegans*. The known vertebrate counterpart to *ced-3* protein is ICE. The overall amino acid identity between *ced-3* and ICE is 28%, with a region of 115 amino acids (residues 246-360 of *ced-3* and 164-278 of ICE) that shows the highest identity (43%). This region contains a conserved pentapeptide, QACRG (residues 356-360 of *ced-3*), which contains a cysteine known to be essential for ICE function. The ICE-LAP-1 and 2 polypeptides of the present invention also have the same conserved pentapeptide and the cysteine residue which is essential for ICE function.

The similarity between *ced-3* and ICE suggests not only that *ced-3* might function as a cysteine protease but also that ICE might act as a vertebrate programmed cell death gene. *ced-3* and the vertebrate counterpart, ICE, control programmed cell death during embryonic development, (Gagliarnini, V. et al., Science, 263:826:828 (1994).

ICE mRNA has been detected in a variety of tissues, including peripheral blood monocytes, peripheral blood lymphocytes, peripheral blood neutrophils, resting and activated peripheral blood T lymphocytes, placenta, the B lymphoblastoid line CB23, and monocytic leukemia cell line THP-1 cells (Cerretti, D.P., et al., Science, 256:97-100 (1992)), suggesting that ICE may have an additional substrate in addition to pro-IL-1β. The substrate that ICE acts upon to cause cell death is presently unknown. One possibility is that it may be a vertebrate homolog of the *C. elegans* cell death gene *ced-4*. Alternatively, ICE might directly cause cell death by proteolytically cleaving proteins that are essential for cell viability.

The mammalian gene *bcl-2*, has been found to protect immune cells called lymphocytes from cell suicide. Also,

crmA, a cow pox virus gene protein product inhibits ICE's protein splitting activity.

In accordance with one aspect of the present invention, there is provided novel mature polypeptides, as well as biologically active and diagnostically or therapeutically useful fragments, analogs and derivatives thereof. The polypeptide of the present invention is of human origin.

In accordance with another aspect of the present invention, there are provided isolated nucleic acid molecules encoding a polypeptide of the present invention including mRNAs, DNAs, cDNAs, genomic DNAs as well as analogs and biologically active and diagnostically or therapeutically useful fragments thereof.

In accordance with yet a further aspect of the present invention, there are provided processes for producing such polypeptides by recombinant techniques comprising culturing recombinant prokaryotic and/or eukaryotic host cells, containing a nucleic acid sequence encoding a polypeptide of the present invention, under conditions promoting expression of said protein and subsequent recovery of said protein.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptides, or polynucleotide encoding such polypeptides for therapeutic purposes, for example, as an antiviral agent, an anti-tumor agent and to control embryonic development and tissue homeostasis.

In accordance with yet a further aspect of the present invention, there is also provided nucleic acid probes comprising nucleic acid molecules of sufficient length to specifically hybridize to a nucleic acid sequence of the present invention.

In accordance with yet a further aspect of the present invention, there are provided antibodies against such polypeptides.

In accordance with yet another aspect of the present invention, there are provided antagonists to such polypeptides, which may be used to inhibit the action of such polypeptides, for example, in the treatment of Alzheimer's disease, Parkinson's disease, rheumatoid arthritis, septic shock and head injury.

In accordance with still another aspect of the present invention, there are provided diagnostic assays for detecting diseases or susceptibility to diseases related to mutations in the nucleic acid sequences encoding a polypeptide of the present invention.

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing such polypeptides, or polynucleotides encoding such polypeptides, for *in vitro* purposes related to scientific research, for example, synthesis of DNA and manufacture of DNA vectors.

These and other aspects of the present invention should be apparent to those skilled in the art from the teachings herein.

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figure 1 shows the cDNA and corresponding deduced amino acid sequence of ICE-LAP-3. The polypeptide encoded by the amino acid sequence shown is the putative mature form of the polypeptide (minus the initial methionine residue), and the standard one-letter abbreviation for amino acids is used.

Figure 2 shows the cDNA and corresponding deduced amino acid sequence of ICE-LAP-4. The polypeptide encoded by the amino acid sequence shown is the putative mature form of the polypeptide (minus the initial methionine residue).

Figure 3 shows an amino acid sequence comparison between ICE-LAP-3, ICE-LAP-4, human ICE and the C. elegan cell death gene ced-3. Shaded areas represent amino acid matches between the different sequences.

In accordance with an aspect of the present invention, there are provided isolated nucleic acids (polynucleotides) which encode the mature polypeptides having the deduced amino acid sequence of Figures 1 and 2 (SEQ ID NO:2 and 4, respectively) or for the mature polypeptide encoded by the cDNA of the clones deposited as ATCC Deposit No. 75875 and 75873. ATCC Deposit No. 75875 contains the cDNA encoding for ICE-LAP-3, and ATCC Deposit No. 75873 contains the cDNA encoding for ICE-LAP-4. The deposit was made August 25, 1994.

The polynucleotide encoding ICE-LAP-3 can be detected from human prostate, human endometrial tumor, human pancreatic tumor, human adrenal gland tumor and human tonsil. The full-length encoding ICE-LAP-3 was discovered in a cDNA library derived from human endometrial tumor. It is structurally related to the Interleukin-1β converting enzyme family. It contains an open reading frame encoding a protein of approximately 341 amino acid residues. The protein exhibits the highest degree of homology to *C. elegans* cell death gene ced-3 which is a hoholog of human interleukin-1β converting enzyme, with 68 % similarity and 43 % identity over the entire amino acid sequence. It should be pointed out that the pentapeptide QACRG is conserved and is located at amino acid position 259-263.

The polynucleotide encoding ICE-LAP-4 was discovered in a cDNA library derived from human tonsils. It is structurally related to the ICE family. It contains an open reading frame encoding a protein of about 277 amino acid residues. The protein exhibits the highest degree of homology to the *C. elegans* cell death gene ced-3 with 29 % identity and 46 % similarity over a 277 amino acid stretch. It is also important that the pentapeptide QACRG is conserved and is located at amino position 161-165.

The polynucleotides of the present invention may be in the form of RNA or in the form of DNA, which DNA includes

cDNA, genomic DNA, and synthetic DNA. The DNA may be double-stranded or single-stranded, and if single stranded may be the coding strand or non-coding (anti-sense) strand. The coding sequence which encode the mature polypeptides may be identical to the coding sequence shown in Figures 1 and 2 (SEQ ID NO:1 and 3) or that of the deposited clones or may be a different coding sequence which coding sequence, as a result of the redundancy or degeneracy of the genetic code, encode the same mature polypeptides, and derivatives thereof, as the DNA of Figures 1 and 2 (SEQ ID NO:1 and 3) or the deposited cDNA.

The polynucleotides which encode for the mature polypeptides of Figures 1 and 2 (SEQ ID NO:2 and 4) or for the mature polypeptides encoded by the deposited cDNAs may include: only the coding sequence for the mature polypeptide; the coding sequence for the mature polypeptide and additional coding sequence; the coding sequence for the mature polypeptide (and optionally additional coding sequence) and non-coding sequence, such as introns or non-coding sequence 5' and/or 3' of the coding sequence for the mature polypeptide.

Thus, the term "polynucleotide encoding a polypeptide" encompasses a polynucleotide which includes only coding sequence for the polypeptide as well as a polynucleotide which includes additional coding and/or non-coding sequence.

The present invention further relates to variants of the hereinabove described polynucleotides which encode for fragments, analogs and derivatives of the polypeptides having the deduced amino acid sequence of Figures 1 and 2 (SEQ ID NO:2 and 4) or the polypeptides encoded by the cDNA of the deposited clones. The variants of the polynucleotides may be naturally occurring allelic variants of the polynucleotides or non-naturally occurring variants of the polynucleotides.

Thus, the present invention includes polynucleotides encoding the same mature polypeptides as shown in Figures 1

and 2 (SEQ ID NO:2 and 4) or the same mature polypeptides encoded by the cDNA of the deposited clones as well as variants of such polynucleotides which variants encode for a fragment, derivative or analog of the polypeptides of Figures 1 and 2 (SEQ ID NO:2 and 4) or the polypeptides encoded by the cDNA of the deposited clones. Such nucleotide variants include deletion variants, substitution variants and addition or insertion variants.

As hereinabove indicated, the polynucleotides may have a coding sequence which is a naturally occurring allelic variant of the coding sequence shown in Figures 1 and 2 (SEQ ID NO:1 and 3) or of the coding sequence of the deposited clones. As known in the art, an allelic variant is an alternate form of a polynucleotide sequence which may have a substitution, deletion or addition of nucleotides, which does not substantially alter the function of the encoded polypeptides. The polynucleotides may also encode for a proprotein which is the mature protein plus additional 5' amino acid residues.

The polynucleotides of the present invention may also have the coding sequence fused in frame to a sequence which allows for purification of the polypeptide of the present invention. The marker sequence may be a hexa-histidine tag supplied by a pQE-9 vector to provide for purification of the mature polypeptides fused to the marker in the case of a bacterial host, or, for example, the marker sequence may be a hemagglutinin (HA) tag when a mammalian host, e.g. COS-7 cells, is used. The HA tag corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson, I., et al., Cell, 37:767 (1984)).

The term "gene" means the segment of DNA involved in producing a polypeptide chain; it includes regions preceding and following the coding region (leader and trailer) as well as intervening sequences (introns) between individual coding segments (exons).

Fragments of the full length genes of the present invention may be used as a hybridization probe for a cDNA library to isolate the full length gene and to isolate other genes which have a high sequence similarity to the gene or similar biological activity. Probes of this type preferably have at least 30 bases and may contain, for example, 50 or more bases. The probe may also be used to identify a cDNA clone corresponding to a full length transcript and a genomic clone or clones that contain the complete gene including regulatory and promotor regions, exons, and introns. An example of a screen comprises isolating the coding region of the gene by using the known DNA sequence to synthesize an oligonucleotide probe. Labeled oligonucleotides having a sequence complementary to that of the gene of the present invention are used to screen a library of human cDNA, genomic DNA or mRNA to determine which members of the library the probe hybridizes to.

The present invention further relates to polynucleotides which hybridize to the hereinabove-described sequences if there is at least 70%, preferably at least 90%, and more preferably at least 95% identity between the sequences. The present invention particularly relates to polynucleotides which hybridize under stringent conditions to the hereinabove-described polynucleotides. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 95% and preferably at least 97% identity between the sequences. The polynucleotides which hybridize to the hereinabove described polynucleotides in a preferred embodiment encode polypeptides which either retain substantially the same biological function or activity as the mature polypeptide encoded by the cDNAs of Figures 1 and 2 (SEQ ID NO:1 and 3) or the deposited cDNA(s).

Alternatively, the polynucleotide may have at least 20 bases, preferably 30 bases, and more preferably at least 50 bases which hybridize to a polynucleotide of the present

invention and which has an identity thereto, as hereinabove described, and which may or may not retain activity. For example, such polynucleotides may be employed as probes for the polynucleotide of SEQ ID NO:1 and 3, for example, for recovery of the polynucleotide or as a diagnostic probe or as a PCR primer.

Thus, the present invention is directed to polynucleotides having at least a 70% identity, preferably at least 90% and more preferably at least a 95% identity to a polynucleotide which encodes the polypeptide of SEQ ID NO:2 and 4 as well as fragments thereof, which fragments have at least 30 bases and preferably at least 50 bases and to polypeptides encoded by such polynucleotides.

The deposit(s) referred to herein will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Micro-organisms for purposes of Patent Procedure. These deposits are provided merely as convenience to those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. §112. The sequence of the polynucleotides contained in the deposited materials, as well as the amino acid sequence of the polypeptides encoded thereby, are incorporated herein by reference and are controlling in the event of any conflict with any description of sequences herein. A license may be required to make, use or sell the deposited materials, and no such license is hereby granted.

The present invention further relates to ICE-LAP-3 and 4 polypeptides which have the deduced amino acid sequence of Figures 1 and 2 (SEQ ID NO:2 and 4) or which has the amino acid sequence encoded by the deposited cDNAs, as well as fragments, analogs and derivatives of such polypeptides.

The terms "fragment," "derivative" and "analog" when referring to the polypeptides of Figures 1 and 2 (SEQ ID NO:2 and 4) or that encoded by the deposited cDNA, means polypeptides which retain essentially the same biological

function or activity as such polypeptides, and wherein derivatives include polypeptides with enhanced or reduced biological function.

The polypeptides of the present invention may be recombinant polypeptides, natural polypeptides or synthetic polypeptides, preferably recombinant polypeptides.

The fragment, derivative or analog of the polypeptides of Figures 1 and 2 (SEQ ID NO:2 and 4) or that encoded by the deposited cDNAs may be (i) one in which one or more of the amino acid residues are substituted with a conserved or non-conserved amino acid residue (preferably a conserved amino acid residue) and such substituted amino acid residue may or may not be one encoded by the genetic code, or (ii) one in which one or more of the amino acid residues includes a substituent group, or (iii) one in which the mature polypeptide is fused with another compound, such as a compound to increase the half-life of the polypeptide (for example, polyethylene glycol), or (iv) one in which the additional amino acids are fused to the mature polypeptide for purification of the mature polypeptide. Such fragments, derivatives and analogs are deemed to be within the scope of those skilled in the art from the teachings herein.

The polypeptides and polynucleotides of the present invention are preferably provided in an isolated form, and preferably are purified to homogeneity. The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated, but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that

such vector or composition is not part of its natural environment.

The polypeptides of the present invention include the polypeptide of SEQ ID NO:2 and 4 (in particular the mature polypeptide) as well as polypeptides which have at least 70% similarity (preferably at leat a 70% identity) to the polypeptide of SEQ ID NO:2 and 4 and more preferably at least a 90% similarity (more preferably at least a 90% identity) to the polypeptide of SEQ ID NO:2 and 4 and still more preferably at least a 95% similarity (still more preferably at least a 95% identity) to the polypeptide of SEQ ID NO:2 and 4 and also include portions of such polypeptides with such portion of the polypeptide generally containing at least 30 amino acids and more preferably at least 50 amino acids.

As known in the art "similarity" between two polypeptides is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one polypeptide to the sequence of a second polypeptide.

Fragments or portions of the polypeptides of the present invention may be employed for producing the corresponding full-length polypeptide by peptide synthesis; therefore, the fragments may be employed as intermediates for producing the full-length polypeptides. Fragments or portions of the polynucleotides of the present invention may be used to synthesize full-length polynucleotides of the present invention.

The present invention also relates to vectors which include polynucleotides of the present invention, host cells which are genetically engineered with vectors of the invention and the production of polypeptides of the invention by recombinant techniques.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the

form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the ICE-LAP-3 and 4 genes. The culture conditions, such as temperature, pH and the like, are those previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

The polynucleotides of the present invention may be employed for producing polypeptides by recombinant techniques. Thus, for example, the polynucleotide may be included in any one of a variety of expression vectors for expressing a polypeptide. Such vectors include chromosomal, nonchromosomal and synthetic DNA sequences, e.g., derivatives of SV40; bacterial plasmids; phage DNA; baculovirus; yeast plasmids; vectors derived from combinations of plasmids and phage DNA, viral DNA such as vaccinia, adenovirus, fowl pox virus, and pseudorabies. However, any other vector may be used as long as it is replicable and viable in the host.

The appropriate DNA sequence may be inserted into the vector by a variety of procedures. In general, the DNA sequence is inserted into an appropriate restriction endonuclease site(s) by procedures known in the art. Such procedures and others are deemed to be within the scope of those skilled in the art.

The DNA sequence in the expression vector is operatively linked to an appropriate expression control sequence(s) (promoter) to direct mRNA synthesis. As representative examples of such promoters, there may be mentioned: LTRs from retroviruses, e.g. RSV, HIV, HTLVI, CMV or SV40 promoter, the E. coli. lac or trp, the phage lambda P_L promoter and other promoters known to control expression of genes in prokaryotic or eukaryotic cells or their viruses. However, also cellular signals can be used, for example, human-β-actin-promoter).

The expression vector can contain a ribosome binding site for translation initiation and a transcription terminator. The vector may also include appropriate sequences for amplifying the copy number of the gene.

In addition, the expression vectors preferably contain one or more selectable marker genes to provide a phenotypic trait for selection of transformed host cells such as dihydrofolate reductase or neomycin resistance for eukaryotic cell culture, or such as tetracycline or ampicillin resistance in E. coli.

The vector containing the appropriate DNA sequence as hereinabove described, as well as an appropriate promoter or control sequence, may be employed to transform an appropriate host to permit the host to express the protein.

As representative examples of appropriate hosts, there may be mentioned: bacterial cells, such as E. coli, Bacillus subtilis, Streptomyces, Salmonella typhimurium; fungal cells, such as yeast; insect cells such as Drosophila and Spodoptera Sf9; adenoviruses; animal cells such as CHO, COS, HEK 293 or Bowes melanoma; plant cells, etc. The selection of an appropriate host is deemed to be within the scope of those skilled in the art from the teachings herein.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available. The following vectors are provided by way of example. Bacterial: pQE70, pQE60, pQE-9 (Qiagen), pbs, pD10, phagescript, psiX174, pbluescript SK, pbsks,

pNH8A, pNH16a, pNH18A, pNH46A (Stratagene); ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 (Pharmacia). Eukaryotic: pWLNEO, pSV2CAT, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, pSVL (Pharmacia). However, any other plasmid or vector may be used as long as they are replicable and viable in the host.

Promoter regions can be selected from any desired gene using CAT (chloramphenicol transferase) vectors or other vectors with selectable markers. Two appropriate vectors are pKK232-8 and pCM7. Particular named bacterial promoters include lacI, lacZ, T3, T7, gpt, lambda P_R, P_L and trp. Eukaryotic promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Selection of the appropriate vector and promoter is well within the level of ordinary skill in the art.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, lipofection or electroporation. (Davis, L., Dibner, M., Battey, I., Basic Methods in Molecular Biology, (1986)).

The constructs in host cells can be used in a conventional manner to produce the gene product encoded by the recombinant sequence. Alternatively, the polypeptides of the invention can be synthetically produced by conventional peptide synthesizers.

Mature proteins can be expressed in mammalian cells, yeast, bacteria, or other cells under the control of appropriate promoters. Cell-free translation systems can also be employed to produce such proteins using RNAs derived

from the DNA constructs of the present invention. Appropriate cloning and expression vectors for use with prokaryotic and eukaryotic hosts are described by Sambrook, et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, N.Y., (1989), the disclosure of which is hereby incorporated by reference.

Transcription of the DNA encoding the polypeptides of the present invention by higher eukaryotes is increased by inserting an enhancer sequence into the vector. Enhancers are cis-acting elements of DNA, usually about from 10 to 300 bp that act on a promoter to increase its transcription. Examples including the SV40 enhancer on the late side of the replication origin bp 100 to 270, a cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

Generally, recombinant expression vectors will include origins of replication and selectable markers permitting transformation of the host cell, e.g., the ampicillin resistance gene of E. coli and S. cerevisiae TRP1 gene, and a promoter derived from a highly-expressed gene to direct transcription of a downstream structural sequence. Such promoters can be derived from operons encoding glycolytic enzymes such as 3-phosphoglycerate kinase (PGK), α-factor, acid phosphatase, or heat shock proteins, among others. The heterologous structural sequence is assembled in appropriate phase with translation initiation and termination sequences, and preferably, a leader sequence capable of directing secretion of translated protein into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product.

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding

a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and to, if desirable, provide amplification within the host. Suitable prokaryotic hosts for transformation include E. coli, Bacillus subtilis, Salmonella typhimurium and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may also be employed as a matter of choice.

As a representative but nonlimiting example, useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, pKK223-3 (Pharmacia Fine Chemicals, Uppsala, Sweden) and GEM1 (Promega Biotec, Madison, WI, USA). These pBR322 "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period.

Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Microbial cells employed in expression of proteins can be disrupted by any convenient method, including freeze-thaw cycling, sonication, mechanical disruption, or use of cell lysing agents, such methods are well know to those skilled in the art.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors may comprise an origin of replication, a suitable promoter and enhancer, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking nontranscribed sequences. DNA sequences derived from the SV40 splice and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The ICE-LAP-3 and 4 polypeptides can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing configuration of the mature protein. Finally, high performance liquid chromatography (HPLC) can be employed for final purification steps.

The polypeptides of the present invention may be a naturally purified product, or a product of chemical synthetic procedures, or produced by recombinant techniques from a prokaryotic or eukaryotic host (for example, by bacterial, yeast, higher plant, insect and mammalian cells in culture). Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. Polypeptides of the invention may also include an initial methionine amino acid residue.

The ICE-LAP-3 and 4 polypeptides may be employed to treat abnormally controlled programmed cell death.

Abnormally controlled programmed cell death may be an underlying cause of cancers due to an abnormal amount of cell growth. Accordingly, since ICE-LAP genes are implicated in programmed cell death, they may be used to target unwanted cells, for example, cancerous cells. ICE-LAP-3 and 4 may also be used to control vertebrate development and tissue homeostasis, due to its apoptosis ability. Also, ICE-LAP-3 and 4 polypeptides may be used to overcome many viral infections by overcoming the suppressed programmed cell death, since programmed cell death may be one of the primary antiviral defense mechanisms of cells.

ICE-LAP-3 and 4 may also be employed to treat immunosuppression related disorders, such as AIDS, by targeting virus infected cells for cell death.

The present invention is further related to a process of screening compounds to identify antagonists to the ICE-LAP-3 and 4 polypeptides of the present invention. An example of such an assay comprises combining ICE-LAP-3 or 4 and a potential antagonist compound with their natural substrate under conditions allowing for action upon the substrate and determining whether the compound prevents ICE-LAP-3 or 4 from cleaving the substrate.

Potential antagonists include an antibody, or in some cases, an oligopeptide, which binds to the polypeptide. Alternatively, a potential antagonist may be a closely related protein which binds to the substrate, however, they are inactive forms of the polypeptide and thereby prevent the action of the polypeptide of the present invention.

Another potential antagonist is an antisense construct prepared using antisense technology. Antisense technology can be used to control gene expression through triple-helix formation or antisense DNA or RNA, both of which methods are based on binding of a polynucleotide to DNA or RNA. For example, the 5' coding portion of the polynucleotide sequence, which encodes for the mature polypeptides of the

present invention, is used to design an antisense RNA oligonucleotide of from about 10 to 40 base pairs in length. A DNA oligonucleotide is designed to be complementary to a region of the gene involved in transcription (triple helix - see Lee et al., Nucl. Acids Res., 6:3073 (1979); Cooney et al, Science, 241:456 (1988); and Dervan et al., Science, 251: 1360 (1991)), thereby preventing transcription and the production of ICE-LAP 3 and 4. The antisense RNA oligonucleotide hybridizes to the mRNA *in vivo* and blocks translation of the mRNA molecule into ICE-LAP 3 and 4 polypeptide (Antisense - Okano, J. Neurochem., 56:560 (1991); Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1988)). The oligonucleotides described above can also be delivered to cells such that the antisense RNA or DNA may be expressed *in vivo* to inhibit production of ICE-LAP 3 and 4.

Potential antagonists include a small molecule which binds to and occupies the catalytic site of the polypeptides thereby making the catalytic site inaccessible to substrate such that normal biological activity is prevented. Examples of small molecules include but are not limited to small peptides or peptide-like molecules.

The antagonists may be employed to treat The antagonists non-programmed necrotic cell death related to cardiovascular diseases, strokes, trauma, and other degenerative diseases where abnormal regulation of ICE-LAP 3 and 4 may lead to pathological cell death, for example, immunosuppression-related disorders, Alzheimer's disease, Parkinson's disease and rheumatoid arthritis.

The antagonists may also be employed to treat immune-based diseases of the lung and airways, central nervous system, eyes and ears, joints, bones, cardiovascular system and gastrointestinal and urogenital systems. The antagonists may be employed in a composition with a pharmaceutically acceptable carrier, e.g., as hereinafter described.

The polypeptides of the present invention and antagonist compounds may be employed in combination with a suitable pharmaceutical carrier. Such compositions comprise a therapeutically effective amount of the polypeptide or antagonist, and a pharmaceutically acceptable carrier or excipient. Such a carrier includes but is not limited to saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The formulation should suit the mode of administration.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the polypeptides or antagonists of the present invention may be employed in conjunction with other therapeutic compounds.

The pharmaceutical compositions may be administered in a convenient manner such as by the intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes. ICE-LAP-3 and 4 are administered in an amount which is effective for treating and/or prophylaxis of the specific indication. In general, ICE-LAP-3 and 4 will be administered in an amount of at least 10 μg/kg body weight, and in most cases they will be administered in an amount not in excess of 8 mg/Kg body weight per day. In most cases, the dosage is from about 10 μg/kg to about 1 mg/kg body weight daily, taking into account the routes of administration, symptoms, etc.

The polypeptides may also be employed in accordance with the present invention by expression of such polypeptides *in vivo*, which is often referred to as "gene therapy."

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) encoding a polypeptide *ex vivo*, with the engineered cells then being provided to a patient to be treated with the polypeptide. Such methods are well-known in the art. For example, cells may be engineered by procedures known in the art by use of a retroviral particle containing RNA encoding a polypeptide of the present invention.

Similarly, cells may be engineered *in vivo* for expression of a polypeptide *in vivo* by, for example, procedures known in the art. As known in the art, a producer cell for producing a retroviral particle containing RNA encoding the polypeptide of the present invention may be administered to a patient for engineering cells *in vivo* and expression of the polypeptide *in vivo*. These and other methods for administering a polypeptide of the present invention by such method should be apparent to those skilled in the art from the teachings of the present invention. For example, the expression vehicle for engineering cells may be other than a retrovirus, for example, an adenovirus which may be used to engineer cells in vivo after combination with a suitable delivery vehicle.

Retroviruses from which the retroviral plasmid vectors hereinabove mentioned may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, adenovirus, Myeloproliferative Sarcoma Virus, and mammary tumor virus. In one embodiment, the retroviral plasmid vector is derived from Moloney Murine Leukemia Virus.

The vector includes one or more promoters. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, et al.,

Biotechniques, Vol. 7, No. 9, 980-990 (1989), or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and β-actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, thymidine kinase (TK) promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

The nucleic acid sequence encoding the polypeptide of the present invention is under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, adenoviral promoters, such as the adenoviral major late promoter; or hetorologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs (including the modified retroviral LTRs hereinabove described); the β-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter which controls the gene encoding the polypeptide.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, ψ-2, ψ-AM, PA12, T19-14X, VT-19-17-H2, ψCRE, ψCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy, Vol. 1, pgs. 5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and $CaPO_4$ precipitation. In one alternative, the

retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include the nucleic acid sequence(s) encoding the polypeptides. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express the nucleic acid sequence(s) encoding the polypeptide. Eukaryotic cells which may be transduced include, but are not limited to, embryonic stem cells, embryonic carcinoma cells, as well as hematopoietic stem cells, hepatocytes, fibroblasts, myoblasts, keratinocytes, endothelial cells, and bronchial epithelial cells.

This invention is also related to the use of the genes of the present invention as a diagnostic. Detection of a mutated form of the genes will allow a diagnosis of a disease or a susceptibility to a disease which results from underexpression of the polypeptide of the present invention.

Individuals carrying mutations in the human ICE-LAP 3 and 4 gene may be detected at the DNA level by a variety of techniques. Nucleic acids for diagnosis may be obtained from a patient's cells, including but not limited to blood, urine, saliva, tissue biopsy and autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR (Saiki *et al.*, Nature, 324:163-166 (1986)) prior to analysis. RNA or cDNA may also be used for the same purpose. As an example, PCR primers complementary to the nucleic acid encoding a polypeptide of the present invention can be used to identify and analyze mutations. For example, deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to radiolabeled ICE-LAP 3 and 4 RNA or alternatively, radiolabeled ICE-LAP 3 and 4 antisense DNA

sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase A digestion or by differences in melting temperatures.

Sequence differences between the reference gene and genes having mutations may be revealed by the direct DNA sequencing method. In addition, cloned DNA segments may be employed as probes to detect specific DNA segments. The sensitivity of this method is greatly enhanced when combined with PCR. For example, a sequencing primer is used with double-stranded PCR product or a single-stranded template molecule generated by a modified PCR. The sequence determination is performed by conventional procedures with radiolabeled nucleotide or by automatic sequencing procedures with fluorescent-tags.

Genetic testing based on DNA sequence differences may be achieved by detection of alteration in electrophoretic mobility of DNA fragments in gels with or without denaturing agents. Small sequence deletions and insertions can be visualized by high resolution gel electrophoresis. DNA fragments of different sequences may be distinguished on denaturing formamide gradient gels in which the mobilities of different DNA fragments are retarded in the gel at different positions according to their specific melting or partial melting temperatures (see, e.g., Myers *et al.*, Science, 230:1242 (1985)).

Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method (e.g., Cotton *et al.*, PNAS, USA, 85:4397-4401 (1985)).

Thus, the detection of a specific DNA sequence may be achieved by methods such as hybridization, RNase protection, chemical cleavage, direct DNA sequencing or the use of restriction enzymes, (e.g., Restriction Fragment Length Polymorphisms (RFLP)) and Southern blotting of genomic DNA.

In addition to more conventional gel-electrophoresis and DNA sequencing, mutations can also be detected by *in situ* analysis.

The present invention also relates to a diagnostic assay for detecting altered levels of ICE-LAP 3 and 4 protein in various tissues. Assays used to detect levels of ICE-LAP 3 and 4 protein in a sample derived from a host are well-known to those of skill in the art and include radioimmunoassays, competitive-binding assays, Western Blot analysis and preferably an ELISA assay. An ELISA assay initially comprises preparing an antibody specific to the ICE-LAP 3 and 4 antigen, preferably a monoclonal antibody. In addition a reporter antibody is prepared against the monoclonal antibody. To the reporter antibody is attached a detectable reagent such as radioactivity, fluorescence or in this example a horseradish peroxidase enzyme. A sample is now removed from a host and incubated on a solid support, e.g. a polystyrene dish, that binds the proteins in the sample. Any free protein binding sites on the dish are then covered by incubating with a non-specific protein such as bovine serum albumin. Next, the monoclonal antibody is incubated in the dish during which time the monoclonal antibodies attach to any ICE-LAP 3 and 4 proteins attached to the polystyrene dish. All unbound monoclonal antibody is washed out with buffer. The reporter antibody linked to horseradish peroxidase is now placed in the dish resulting in binding of the reporter antibody to any monoclonal antibody bound to ICE-LAP 3 and 4. Unattached reporter antibody is then washed out. Peroxidase substrates are then added to the dish and the amount of color developed in a given time period is a measurement of the amount of ICE-LAP 3 and 4 protein present in a given volume of patient sample when compared against a standard curve.

A competition assay may be employed wherein antibodies specific to ICE-LAP 3 and 4 are attached to a solid support

and labeled ICE-LAP 3 and 4 and a sample derived from the host are passed over the solid support and the amount of label detected attached to the solid support can be correlated to a quantity of ICE-LAP 3 and 4 in the sample.

The sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. Moreover, there is a current need for identifying particular sites on the chromosome. Few chromosome marking reagents based on actual sequence data (repeat polymorphisms) are presently available for marking chromosomal location. The mapping of DNAs to chromosomes according to the present invention is an important first step in correlating those sequences with genes associated with disease.

Briefly, sequences can be mapped to chromosomes by preparing PCR primers (preferably 15-25 bp) from the cDNA. Computer analysis of the 3' untranslated region of the gene is used to rapidly select primers that do not span more than one exon in the genomic DNA, thus complicating the amplification process. These primers are then used for PCR screening of somatic cell hybrids containing individual human chromosomes. Only those hybrids containing the human gene corresponding to the primer will yield an amplified fragment.

PCR mapping of somatic cell hybrids is a rapid procedure for assigning a particular DNA to a particular chromosome. Using the present invention with the same oligonucleotide primers, sublocalization can be achieved with panels of fragments from specific chromosomes or pools of large genomic clones in an analogous manner. Other mapping strategies that can similarly be used to map to its chromosome include *in situ* hybridization, prescreening with labeled flow-sorted chromosomes and preselection by hybridization to construct chromosome specific-cDNA libraries.

Fluorescence *in situ* hybridization (FISH) of a cDNA clone to a metaphase chromosomal spread can be used to provide a precise chromosomal location in one step. This technique can be used with cDNA having at least 50 or 60 bases. For a review of this technique, see Verma et al., Human Chromosomes: a Manual of Basic Techniques, Pergamon Press, New York (1988).

Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

Next, it is necessary to determine the differences in the cDNA or genomic sequence between affected and unaffected individuals. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

With current resolution of physical mapping and genetic mapping techniques, a cDNA precisely localized to a chromosomal region associated with the disease could be one of between 50 and 500 potential causative genes. (This assumes 1 megabase mapping resolution and one gene per 20 kb).

The polypeptides, their fragments or other derivatives, or analogs thereof, or cells expressing them can be used as an immunogen to produce antibodies thereto. These antibodies can be, for example, polyclonal or monoclonal antibodies. The present invention also includes chimeric, single chain, and humanized antibodies, as well as Fab fragments, or the product of an Fab expression library. Various procedures

known in the art may be used for the production of such antibodies and fragments.

Antibodies generated against the polypeptides corresponding to a sequence of the present invention can be obtained by direct injection of the polypeptides into an animal or by administering the polypeptides to an animal, preferably a nonhuman. The antibody so obtained will then bind the polypeptides itself. In this manner, even a sequence encoding only a fragment of the polypeptides can be used to generate antibodies binding the whole native polypeptides. Such antibodies can then be used to isolate the polypeptide from tissue expressing that polypeptide.

For preparation of monoclonal antibodies, any technique which provides antibodies produced by continuous cell line cultures can be used. Examples include the hybridoma technique (Kohler and Milstein, 1975, Nature, 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., 1983, Immunology Today 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole, et al., 1985, in Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96).

Techniques described for the production of single chain antibodies (U.S. Patent 4,946,778) can be adapted to produce single chain antibodies to immunogenic polypeptide products of this invention. Also, transgenic mice may be used to express humanized antibodies to immunogenic polypeptide products of this invention.

The present invention will be further described with reference to the following examples; however, it is to be understood that the present invention is not limited to such examples. All parts or amounts, unless otherwise specified, are by weight.

In order to facilitate understanding of the following examples certain frequently occurring methods and/or terms will be described.

"Plasmids" are designated by a lower case p preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

"Digestion" of DNA refers to catalytic cleavage of the DNA with a restriction enzyme that acts only at certain sequences in the DNA. The various restriction enzymes used herein are commercially available and their reaction conditions, cofactors and other requirements were used as would be known to the ordinarily skilled artisan. For analytical purposes, typically 1 μg of plasmid or DNA fragment is used with about 2 units of enzyme in about 20 μl of buffer solution. For the purpose of isolating DNA fragments for plasmid construction, typically 5 to 50 μg of DNA are digested with 20 to 250 units of enzyme in a larger volume. Appropriate buffers and substrate amounts for particular restriction enzymes are specified by the manufacturer. Incubation times of about 1 hour at 37°C are ordinarily used, but may vary in accordance with the supplier's instructions. After digestion the reaction is electrophoresed directly on a polyacrylamide gel to isolate the desired fragment.

Size separation of the cleaved fragments is performed using 8 percent polyacrylamide gel described by Goeddel, D. *et al.*, Nucleic Acids Res., 8:4057 (1980), or agarose gels (0.5 - 1.5%).

"Oligonucleotides" refers to either a single stranded polydeoxynucleotide or two complementary polydeoxynucleotide strands which may be chemically synthesized. Such synthetic oligonucleotides have no 5' phosphate and thus will not ligate to another oligonucleotide without adding a phosphate

with an ATP in the presence of a kinase. A synthetic oligonucleotide will ligate to a fragment that has not been dephosphorylated.

"Ligation" refers to the process of forming phosphodiester bonds between two double stranded nucleic acid fragments (Maniatis, T., et al., Id., p. 146). Unless otherwise provided, ligation may be accomplished using known buffers and conditions with 10 units to T4 DNA ligase ("ligase") per 0.5 μg of approximately equimolar amounts of the DNA fragments to be ligated.

Unless otherwise stated, transformation was performed as described in the method of Graham, F. and Van der Eb, A., Virology, 52:456-457 (1973).

Example 1

Bacterial Expression and Purification of ICE-LAP-3

The DNA sequence encoding ICE-LAP-3, ATCC # 75875, is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the processed ICE-LAP-3 protein (minus the signal peptide sequence) and the vector sequences 3' to the ICE-LAP-3 gene. Additional nucleotides corresponding to ICE-LAP-3 are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' GATCGGATCCATGCGTGCGGGGACACGGGTC 3' (SEQ ID NO:5) contains a Bam HI restriction enzyme site (underlined) followed by 18 nucleotides of ICE-LAP-3 coding sequence starting from the presumed terminal amino acid of the processed protein codon. The 3' sequence 5' GTACTCTAGATCATTCACCCTGGTGGAGGAT 3' (SEQ ID NO:6) contains complementary sequences to an Xba I site (underlined) followed by 21 nucleotides of ICE-LAP-3. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). pQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an

IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-9 is then digested with Bam HI and Xba I. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform E. coli available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 ($O.D.^{600}$) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized ICE-LAP-3 is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). ICE-LAP-3 (95 % pure is eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar glutathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in

this solution for 12 hours the protein was dialyzed to 10 mmolar sodium phosphate.

Example 2

Bacterial Expression and Purification of ICE-LAP-4

The DNA sequence encoding ICE-LAP-4, ATCC # 75873, is initially amplified using PCR oligonucleotide primers corresponding to the 5' sequences of the processed ICE-LAP-4 protein (minus the signal peptide sequence) and the vector sequences 3' to the ICE-LAP-4 gene. Additional nucleotides corresponding to ICE-LAP-4 are added to the 5' and 3' sequences respectively. The 5' oligonucleotide primer has the sequence 5' GATCGGATCCATGGAGAACACTGAAAACTCA 3' (SEQ ID NO:7) contains a Bam HI restriction enzyme site (underlined) followed by 18 nucleotides of ICE-LAP-4 coding sequence starting from the presumed terminal amino acid of the processed protein codon. The 3' sequence 5' GTACTCTAGATTAGTGATAAAAATAGAGTTC 3' (SEQ ID NO:8) contains complementary sequences to an Xba I site (underlined) followed by 21 nucleotides of ICE-LAP-4. The restriction enzyme sites correspond to the restriction enzyme sites on the bacterial expression vector pQE-9 (Qiagen, Inc. 9259 Eton Avenue, Chatsworth, CA, 91311). pQE-9 encodes antibiotic resistance (Amp^r), a bacterial origin of replication (ori), an IPTG-regulatable promoter operator (P/O), a ribosome binding site (RBS), a 6-His tag and restriction enzyme sites. pQE-9 is then digested with Bam HI and Xba I. The amplified sequences are ligated into pQE-9 and are inserted in frame with the sequence encoding for the histidine tag and the RBS. The ligation mixture is then used to transform E. coli available from Qiagen under the trademark M15/rep 4 by the procedure described in Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989). M15/rep4 contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers

kanamycin resistance (Kan^r). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis. Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 ($O.D.^{600}$) of between 0.4 and 0.6. IPTG ("Isopropyl-B-D-thiogalacto pyranoside") is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression. Cells are grown an extra 3 to 4 hours. Cells are then harvested by centrifugation. The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl. After clarification, solubilized ICE-LAP-4 is purified from this solution by chromatography on a Nickel-Chelate column under conditions that allow for tight binding by proteins containing the 6-His tag (Hochuli, E. et al., J. Chromatography 411:177-184 (1984)). ICE-LAP-4 (95 % pure is eluted from the column in 6 molar guanidine HCl pH 5.0 and for the purpose of renaturation adjusted to 3 molar guanidine HCl, 100mM sodium phosphate, 10 mmolar glutathione (reduced) and 2 mmolar glutathione (oxidized). After incubation in this solution for 12 hours the protein was dialyzed to 10 mmolar sodium phosphate.

Example 3

Expression of Recombinant ICE-LAP-3 in COS cells

The expression of a plasmid, ICE-LAP-3 HA, is derived from a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CMV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. A DNA fragment encoding the entire ICE-LAP-3 precursor and a

HA tag fused in frame to its 3' end was cloned into the polylinker region of the vector, therefore, the recombinant protein expression is directed under the CMV promoter. The HA tag correspond to an epitope derived from the influenza hemagglutinin protein as previously described (I. Wilson, H. Niman, R. Heighten, A Cherenson, M. Connolly, and R. Lerner, 1984, Cell 37, 767). The infusion of HA tag to our target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is described as follows:

The DNA sequence encoding for ICE-LAP-3, ATCC # 75875, was constructed by PCR on the full-length ICE-LAP-3 using two primers: the 5' primer 5' GACTATGCGTGCGGGGACACGG 3' (SEQ ID NO:9) contains the ICE-LAP-3 translational initiation site ATG followed by 5 nucleotides of ICE-LAP-3 coding sequence starting from the initiation codon; the 3' sequence 5' AATCAAGCGTAGTCTGGGACGTCGTATGGGTATTCACCCTGGTGGAGGATTTG3' (SEQ ID NO:10) contains translation stop codon, HA tag and the last 21 nucleotides of the ICE-LAP-3 coding sequence (not including the stop codon). Therefore, the PCR product contains the ICE-LAP-3 coding sequence followed by HA tag fused in frame, and a translation termination stop codon next to the HA tag. The PCR amplified DNA fragment was ligated with pcDNAI/Amp by blunt end ligation. The ligation mixture was transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant ICE-LAP-3, COS cells were transfected with the expression vector by DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring

Laboratory Press, (1989)). The expression of the ICE-LAP-3 HA protein was detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with ^{35}S-cysteine two days post transfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5) (Wilson, I. et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.

Example 4

Expression of Recombinant ICE-LAP-4 in COS cells

The expression of a plasmid, ICE-LAP-4 HA, is derived from a vector pcDNAI/Amp (Invitrogen) containing: 1) SV40 origin of replication, 2) ampicillin resistance gene, 3) E.coli replication origin, 4) CMV promoter followed by a polylinker region, a SV40 intron and polyadenylation site. A DNA fragment encoding the entire ICE-LAP-4 precursor and a HA tag fused in frame to its 3' end was cloned into the polylinker region of the vector, therefore, the recombinant protein expression is directed under the CMV promoter. The HA tag correspond to an epitope derived from the influenza hemagglutinin protein as previously described (I. Wilson, H. Niman, R. Heighten, A Cherenson, M. Connolly, and R. Lerner, 1984, Cell 37, 767). The infusion of HA tag to our target protein allows easy detection of the recombinant protein with an antibody that recognizes the HA epitope.

The plasmid construction strategy is described as follows:

The DNA sequence encoding for ICE-LAP-4, ATCC # 75873, was constructed by PCR on the full-length ICE-LAP-4 using two primers: the 5' primer 5' ACCATGGAGAACACTGAAAAC 3' (SEQ ID

NO:11) contains the ICE-LAP-4 translational initiation site, ATG, followed by 15 nucleotides of ICE-LAP-4 coding sequence starting from the initiation codon; the 3' sequence 5' AATCAAGCGTAGTCTGGGACGTCGTATGGGTAGTGATAAAAATAGAGTTCTTT 3' (SEQ ID NO:12) contains translation stop codon, HA tag and the last 21 nucleotides of the ICE-LAP-4 coding sequence (not including the stop codon). Therefore, the PCR product contains the ICE-LAP-4 coding sequence followed by HA tag fused in frame, and a translation termination stop codon next to the HA tag. The PCR amplified DNA fragment was ligated with pcDNAI/Amp by blunt end ligation. The ligation mixture was transformed into E. coli strain SURE (available from Stratagene Cloning Systems, 11099 North Torrey Pines Road, La Jolla, CA 92037) the transformed culture was plated on ampicillin media plates and resistant colonies were selected. Plasmid DNA was isolated from transformants and examined by restriction analysis for the presence of the correct fragment. For expression of the recombinant ICE-LAP-4, COS cells were transfected with the expression vector by the DEAE-DEXTRAN method (J. Sambrook, E. Fritsch, T. Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Laboratory Press, (1989)). The expression of the ICE-LAP-4 HA protein was detected by radiolabelling and immunoprecipitation method (E. Harlow, D. Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, (1988)). Cells were labelled for 8 hours with ^{35}S-cysteine two days post transfection. Culture media were then collected and cells were lysed with detergent (RIPA buffer (150 mM NaCl, 1% NP-40, 0.1% SDS, 1% NP-40, 0.5% DOC, 50mM Tris, pH 7.5) (Wilson, I. et al., Id. 37:767 (1984)). Both cell lysate and culture media were precipitated with a HA specific monoclonal antibody. Proteins precipitated were analyzed on 15% SDS-PAGE gels.

Example 5

Expression pattern of ICE-LAP-3 in human tissue

Northern blot analysis was carried out to examine the levels of expression of ICE-LAP-3 in human tissues. Total cellular RNA samples were isolated with RNAzol™ B system (Biotecx Laboratories, Inc. 6023 South Loop East, Houston, TX 77033). About 10μg of total RNA isolated from each human tissue specified was separated on 1% agarose gel and blotted onto a nylon filter (Sambrook, Fritsch, and Maniatis, Molecular Cloning, Cold Spring Harbor Press, (1989)). The labeling reaction was done according to the Stratagene Prime-It kit with 50ng DNA fragment. The labeled DNA was purified with a Select-G-50 column. (5 Prime - 3 Prime, Inc. 5603 Arapahoe Road, Boulder, CO 80303). The filter was then hybridized with radioactive labeled full length ICE-LAP-3 gene at 1,000,000 cpm/ml in 0.5 M $NaPO_4$, pH 7.4 and 7% SDS overnight at 65°C. After wash twice at room temperature and twice at 60°C with 0.5 x SSC, 0.1% SDS, the filter was then exposed at -70°C overnight with an intensifying screen. The message RNA for ICE-LAP-3 is abundant in liver.

Example 6

Expression pattern of ICE-LAP-4 in human tissue

Northern blot analysis is carried out to examine the levels of expression of ICE-LAP-4 in human tissues. Total cellular RNA samples were isolated with RNAzol™ B system (Biotecx Laboratories, Inc. 6023 South Loop East, Houston, TX 77033). About 10μg of total RNA isolated from each human tissue specified was separated on 1% agarose gel and blotted onto a nylon filter (Sambrook, Fritsch, and Maniatis, Molecular Cloning, Cold Spring Harbor Press, (1989)). The labeling reaction was done according to the Stratagene Prime-It kit with 50ng DNA fragment. The labeled DNA was purified with a Select-G-50 column. (5 Prime - 3 Prime, Inc. 5603 Arapahoe Road, Boulder, CO 80303). The filter was then hybridized with radioactive labeled full length ICE-LAP-4 gene at 1,000,000 cpm/ml in 0.5 M $NaPO_4$, pH 7.4 and 7% SDS

overnight at 65°C. After wash twice at room temperature and twice at 60°C with 0.5 x SSC, 0.1% SDS, the filter was then exposed at -70°C overnight with an intensifying screen.

Example 5

Expression via Gene Therapy

Fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask, approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10% FBS, penicillin and streptomycin, is added. This is then incubated at 37°C for approximately one week. At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

pMV-7 (Kirschmeier, P.T. et al, DNA, 7:219-25 (1988) flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated on agarose gel and purified, using glass beads.

The cDNA encoding a polypeptide of the present invention is amplified using PCR primers which correspond to the 5' and 3' end sequences respectively. The 5' primer containing an EcoRI site and the 3' primer further includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate

for ligation of the two fragments. The ligation mixture is used to transform bacteria HB101, which are then plated onto agar-containing kanamycin for the purpose of confirming that the vector had the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells are transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his.

The engineered fibroblasts are then injected into the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads. The fibroblasts now produce the protein product.

Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, within the scope of the appended claims, the invention may be practiced otherwise than as particularly described.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: HE, ET AL.

(ii) TITLE OF INVENTION: Interleukin-1β Converting Enzyme Like Apoptosis Protease- 3 and 4

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:

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(E) COUNTRY: USA
(F) ZIP: 07068

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5 INCH DISKETTE
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: MS-DOS
(D) SOFTWARE: WORD PERFECT 5.1

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE: submitted herewith
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA

(A) APPLICATION NUMBER: 08/334,251
(B) FILING DATE: 11/1/94

(viii) ATTORNEY/AGENT INFORMATION:
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(B) REGISTRATION NUMBER: 36,134
(C) REFERENCE/DOCKET NUMBER: 325800-???

(ix) TELECOMMUNICATION INFORMATION:
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(B) TELEFAX: 201-994-1744

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 1371 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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GGCCAACTTG GCAGAGCGCG CGGCCAGCTT TGCAGAGAGC GCCCTCCAGG GACTATGCGT    120
GCGGGGACAC GGGTCGCTTT GGGCTCTTCC ACCCCTGCGG AGCGCACTAC CCCGAGCCAG    180
GGGCGGTGCA AGCCCCGCCC GGCCCTACCC AGGGCGGCTC CTCCCTCCGC AGCGCCGAGA    240
CTTTTAGTTT CGCTTTCGCT AAAGGGGCCC CAGACCCTTG CTGCGGAGCG ACGGAGAGAG    300
ACTGTGCCAG TCCCAGCCGC CCTACCGCCG TGGGAACGAT GGCAGATGAT TCAGGGCTGT    360
ATTGAAGAGC AGGGGGTTGA GGATTCAGCA AATGAAGATT CAGTGGATGC TAAGCCAGAC    420
CGGTCCTCGT TTGTACCGTC CCTCTTCAGT AAGAAGAAGA AAAATGTCAC CATGCGATCC    480
ATCAAGACCA CCCGGGACCG AGTGCCTACA TATCAGTACA ACATGAATTT TGAAAAGCTG    540
GGCAAATGCA TCATAATAAA CAACAAGAAC TTTGATAAAG TGACAGGTAT GGGCGTTCGA    600
AACGGAACAG ACAAAGATGC CGAGGCGCTC TTCAAGTGCT TCCGAAGCCT GGGTTTTGAC    660
GTGATTGTCT ATAATGACTG CTCTTGTGCC AAGATGCAAG ATCTGCTTAA AAAAGCTTCT    720
GAAGAGGACC ATACAAATGC CGCCTGCTTC GCCTGCATCC TCTTAAGCCA TGGAGAAGAA    780
AATGTAATTT ATGGGAAAGA TGGTGTCACA CCAATAAAGG ATTTGACAGC CCACTTTAGG    840
GGGGATAGAT GCAAAACCCT TTTAGAGAAA CCCAAACTCT TCTTCATTCA GGCTTGCCGA    900
GGGACCGAGC TTGATGATGG CATCCAGGCC GACTCGGGGC CCATCAATGA CACAGATGCT    960
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AATCCTCGAT ACAAGATCCC AGTGGAAGCT GACTTCCTCT TCGCCTATTC CACGGTTCCA   1020
GGCTATTACT CGTGGAGGAG CCCAGGAAGA GGCTCCTGGT TTGTGCAAGC CCTCTGCTCC   1080
ATCCTGGAGG AGCACGGAAA AGACCTGGAA ATCATGCAAA TCCTCCACCA GGGTGAATGA   1140
CAGAGTTGCC AGGCACTTTG AGTCTCAGTC TGATGACCCA CACTTCCATG AGAAGAAGCA   1200
GATCCCCTGT GTGGTCTCCA TGCTCACCAA GGAACTCTAC TTCAGTCAAT AGCCATATCA   1260
GGGGTACATT CTAGCTGAGA AGCAATGGGT CACTCATTAA TGAATCACAT TTTTTTATGC   1320
TCTTGAAATA TTCAGAAATT CTCCAGGATT TTAATTTCAG GAAAATGTAT T             1371
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(3) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 341 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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Glu Arg Thr Thr Pro Ser Gln Gly Arg Cys Lys Pro Arg Pro Ala
            20                  25                  30
Leu Pro Arg Ala Ala Pro Pro Ser Ala Ala Pro Arg Leu Leu Val
        35                  40                  45
Ser Leu Ser Leu Lys Gly Pro Gln Thr Leu Ala Ala Glu Arg Arg
    50                  55                  60
Arg Glu Thr Val Pro Val Pro Ala Ala Leu Pro Pro Trp Glu Arg
65                  70                  75
Thr Gln Met Ile Gln Gly Cys Ile Glu Glu Gln Gly Val Glu Asp
                80                  85                  90
Ser Ala Asn Glu Asp Ser Val Asp Ala Lys Pro Asp Arg Ser Ser
            95                  100                 105
Phe Val Pro Ser Leu Phe Ser Lys Lys Lys Lys Asn Val Thr Met
        110                 115                 120
Arg Ser Ile Lys Thr Thr Arg Asp Arg Val Pro Thr Tyr Gln Tyr
```

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                125                 130                 135
Asn Met Asn Phe Glu Lys Leu Gly Lys Cys Ile Ile Ile Asn Asn
                140                 145                 150
Lys Asn Phe Asp Lys Val Thr Gly Met Gly Val Arg Asn Gly Thr
                155                 160                 165
Asp Lys Asp Ala Glu Ala Leu Phe Lys Cys Phe Arg Ser Leu Gly
                170                 175                 180
Phe Asp Val Ile Val Tyr Asn Asp Cys Ser Cys Ala Lys Met Gln
                185                 190                 195
Asp Leu Leu Lys Lys Ala Ser Glu Glu Asp His Thr Asn Ala Ala
                200                 205                 210
Cys Phe Ala Cys Ile Leu Leu Ser His Gly Glu Glu Asn Val Ile
                215                 220                 225
Tyr Gly Lys Asp Gly Val Thr Pro Ile Lys Asp Leu Thr Ala His
                230                 235                 240
Phe Arg Gly Asp Arg Cys Lys Thr Leu Leu Glu Lys Pro Lys Leu
                245                 250                 255
Phe Phe Ile Gln Ala Cys Arg Gly Thr Glu Leu Asp Asp Gly Ile
                260                 265                 270
Gln Ala Asp Ser Gly Pro Ile Asn Asp Thr Asp Ala Asn Pro Arg
                275                 280                 285
Tyr Lys Ile Pro Val Glu Ala Asp Phe Leu Phe Ala Tyr Ser Thr
                290                 295                 300
Val Pro Gly Tyr Tyr Ser Trp Arg Ser Pro Gly Arg Gly Ser Trp
                305                 310                 315
Phe Val Gln Ala Leu Cys Ser Ile Leu Glu Glu His Gly Lys Asp
                320                 325                 330
Leu Glu Ile Met Gln Ile Leu His Gln Gly Glu
                335                 340
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(4) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 1159 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GCACGAGCGG ATGGGTGCTA TTGTGAGGCG GTTGTAGAAG AGTTTCGTGA GTGCTCGCAG     60
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GCCGTGAGGA GTTAGCGAGC CCTGCTCACA CTCGGCGCTC TGGTTTTCGG TGGGTGTGCC    180
CTGCACCTGC CTCTTCCCGC ATTCTCATTA ATAAAGGTAT CCATGGAGAA CACTGAAAAC    240
TCAGTGGATT CAAAATCCAT TAAAAATTTG GAACCAAAGA TCATACATGG AAGCGAATCA    300
ATGGACTCTG GAATATCCCT GGACAACAGT TATAAAATGG ATTATCCTGA GATGGGTTTA    360
TGTATAATAA TTAATAATAA GAATTTTCAT AAAAGCACTG GAATGACATC TCGGTCTGGT    420
ACAGATGTCG ATGCAGCAAA CCTCAGGGAA ACATTCAGAA ACTTGAAATA TGAAGTCAGG    480
AATAAAAATG ATCTTACACG TGAAGAAATT GTGGAATTGA TGCGTGATGT TTCTAAAGAA    540
GATCACAGCA AAAGGAGCAG TTTTGTTTGT GTGCTTCTGA GCCATGGTGA AGAAGGAATA    600
ATTTTTGGAA CAAATGGACC TGTTGACCTG AAAAAAATAA CAAACTTTTT CAGAGGGGAT    660
CGTTGTAGAA GTCTAACTGG AAAACCCAAA CTTTTCATTA TTCAGGCCTG CCGTGGTACA    720
GAACTGGACT GTGGCATTGA GACAGACAGT GGTGTTGATG ATGACATGGC GTGTCATAAA    780
ATACCAGTGG AGGCCGACTT CTTGTATGCA TACTCCACAG CACCTGGTTA TTATTCTTGG    840
CGAAATTCAA AGGATGGCTC CTGGTTCATC CAGTCGCTTT GTGCCATGCT GAAACAGTAT    900
GCCGACAAGC TTGAATTTAT GCACATTCTT ACCCGGGTTA ACCGAAAGGT GGCAACAGAA    960
TTTGAGTCCT TTTCCTTTGA CGCTACTTTT CATGCAAAGA AACAGATTCC ATGTATTGTT   1020
TCCATGCTCA CAAAAGAACT CTATTTTTAT CACTAAAGAA ATGGTTGGTT GGTGGTTTTT   1080
TTTAGTTTGT ATGCCAAGTG AGAAGATGGT ATATTTGGGT ACTGTATTTC CCTCTCATTG   1140
GGGACCTACT CTCATGCTG                                                1159
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(5) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 277 AMINO ACIDS

(B) TYPE: AMINO ACID

(C) STRANDEDNESS:

(D) TOPOLOGY: LINEAR 2

(ii) MOLECULE TYPE: PROTEIN

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Glu Asn Thr Glu Asn Ser Val Asp Ser Lys Ser Ile Lys Asn
5 10 15
Leu Glu Pro Lys Ile Ile His Gly Ser Glu Ser Met Asp Ser Gly
20 25 30
Ile Ser Leu Asp Asn Ser Tyr Lys Met Asp Tyr Pro Glu Met Gly
35 40 45
Leu Cys Ile Ile Ile Asn Asn Lys Asn Phe His Lys Ser Thr Gly
50 55 60
Met Thr Ser Arg Ser Gly Thr Asp Val Asp Ala Ala Asn Leu Arg
65 70 75
Glu Thr Phe Arg Asn Leu Lys Tyr Glu Val Arg Asn Lys Asn Asp
80 85 90
Leu Thr Arg Glu Glu Ile Val Glu Leu Met Arg Asp Val Ser Lys
95 100 105
Glu Asp His Ser Lys Arg Ser Ser Phe Val Cys Val Leu Leu Ser
110 115 120
His Gly Glu Glu Gly Ile Ile Phe Gly Thr Asn Gly Pro Val Asp
125 130 135
Leu Lys Lys Ile Thr Asn Phe Phe Arg Gly Asp Arg Cys Arg Ser
140 145 150
Leu Thr Gly Lys Pro Lys Leu Phe Ile Ile Gln Ala Cys Arg Gly
155 160 165
Thr Glu Leu Asp Cys Gly Ile Glu Thr Asp Ser Gly Val Asp Asp
170 175 180
Asp Met Ala Cys His Lys Ile Pro Val Glu Ala Asp Phe Leu Tyr
185 190 195
Ala Tyr Ser Thr Ala Pro Gly Tyr Tyr Ser Trp Arg Asn Ser Lys
200 205 210
Asp Gly Ser Trp Phe Ile Gln Ser Leu Cys Ala Met Leu Lys Gln
215 220 225
Tyr Ala Asp Lys Leu Glu Phe Met His Ile Leu Thr Arg Val Asn
230 235 240
Arg Lys Val Ala Thr Glu Phe Glu Ser Phe Ser Phe Asp Ala Thr

245 250 255

Phe His Ala Lys Lys Gln Ile Pro Cys Ile Val Ser Met Leu Thr

260 265 270

Lys Glu Leu Tyr Phe Tyr His

275

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 31 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATCGGATCC ATGCGTGCGG GGACACGGGT C 31

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 31 BASE PAIRS

(B) TYPE: NUCLEIC ACID

(C) STRANDEDNESS: SINGLE

(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTACTCTAGA TCATTCACCC TGGTGGAGGA T 31

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS

(A) LENGTH: 31 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
GATCGGATCC ATGGAGAACA CTGAAAACTC A 31

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 31 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:
GTACTCTAGA TTAGTGATAA AAATAGAGTT C 31

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 22 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:
GACTATGCGT GCGGGGACAC GG 22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 53 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:
AATCAAGCGT AGTCTGGGAC GTCGTATGGG TATTCACCCT GGTGGAGGAT TTG 53

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 21 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:
ACCATGGAGA ACACTGAAAA C 21

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS
(A) LENGTH: 53 BASE PAIRS
(B) TYPE: NUCLEIC ACID
(C) STRANDEDNESS: SINGLE
(D) TOPOLOGY: LINEAR

(ii) MOLECULE TYPE: Oligonucleotide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AATCAAGCGT AGTCTGGGAC GTCGTATGGG TAGTGATAAA AATAGAGTTC TTT 53

WHAT IS CLAIMED IS:

1. An isolated polynucleotide comprising a member selected from the group consisting of:

(a) a polynucleotide encoding the polypeptide comprising amino acid 1 to amino acid 341 as set forth in SEQ ID NO:2;

(b) a polynucleotide encoding the polypeptide comprising amino acid 1 to amino acid 277 as set forth in SEQ ID NO:4;

(c) a polynucleotide capable of hybridizing to and which is at least 95% identical to the polynucleotide of (a) or (b); and

(d) a polynucleotide fragment of the polynucleotide of (a), (b) or (c).

2. The polynucleotide of Claim 1 wherein the polynucleotide is DNA.

3. The polynucleotide of Claim 1 wherein the polynucleotide is RNA.

4. The polynucleotide of Claim 1 wherein the polynucleotide is genomic DNA.

5. An isolated polynucleotide comprising a member selected from the group consisting of:

(a) a polynucleotide which encodes a mature polypeptide having the amino acid sequence expressed by the DNA contained in ATCC Deposit No. 75875;

(b) a polynucleotide which encodes a mature polypeptide having the amino acid sequence expressed by the DNA contained in ATCC Deposit No. 75873;

(c) a polynucleotide capable of hybridizing to and which is at least 95% identical to the polynucleotide of (a); and

(d) a polynucleotide fragment of the polynucleotide of (a), (b) or (c).

6. A vector containing the DNA of Claim 2.

7. A host cell genetically engineered with the vector of Claim 6.

8. A process for producing a polypeptide comprising: expressing from the host cell of Claim 7 the polypeptide encoded by said DNA.

9. A process for producing cells capable of expressing a polypeptide comprising genetically engineering cells with the vector of Claim 6.

10. A polypeptide selected from the group consisting of (i) a polypeptide having the deduced amino acid sequence of SEQ ID NO:2 and fragments, analogs and derivatives thereof; (ii) a polypeptide encoded by the cDNA of ATCC Deposit No. 75875 and fragments, analogs and derivatives of said polypeptide; (iii) a polypeptide having the deduced amino acid sequence of SEQ ID NO:4 and fragments, analogs and derivatives thereof; and (iv) a polypeptide encoded by the cDNA of ATCC Deposit No. 75873 and fragments, analogs and derivatives of said polypeptide.

11. A compound which inhibits acitivation of the polypeptide of claim 10.

12. A method for the treatment of a patient having need of ICE-LAP-3 comprising: administering to the patient a therapeutically effective amount of the polypeptide of claim 10.

13. The method of Claim 12 wherein said therapeutically effective amount of the polypeptide is administered by providing to the patient DNA encoding said polypeptide and expressing said polypeptide *in vivo*.

14. A method for the treatment of a patient having need of ICE-LAP-4 comprising: administering to the patient a therapeutically effective amount of the polypeptide of claim 10.

15. The method of Claim 14 wherein said therapeutically effective amount of the polypeptide is administered by providing to the patient DNA encoding said polypeptide and expressing said polypeptide *in vivo*.

16. A method for the treatment of a patient having need to inhibit an ICE-LAP 3 polypeptide comprising: administering to the patient a therapeutically effective amount of the compound of Claim 11.

17. A method for the treatment of a patient having need to inhibit an ICE-LAP 4 polypeptide comprising: administering to the patient a therapeutically effective amount of the compound of Claim 11.

18. A process for diagnosing a disease or a susceptibility to a disease related to an under-expression of the polypeptide of claim 10 comprising:

determining a mutation in a nucleic acid sequence encoding said polypeptide.

19. A diagnostic process comprising:

analyzing for the presence of the polypeptide of claim 10 in a sample derived from a host.

20. A method for identifying compounds which inhibit the polypeptide of claim 10 comprising:

contacting the polypeptide with its natural substrate and a compound under conditions where the substrate is normally cleaved by the polypeptide; and

determining whether the compound inhibits the polypeptide by detecting the absence of cleaved substrate.

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ABSTRACT OF THE DISCLOSURE

Disclosed are human interleukin-1 β converting enzyme like apoptosis proteases-3 and 4 and DNA (RNA) encoding such polypeptides. Also provided is a procedure for producing such polypeptides by recombinant techniques and antibodies and antagonists against such polypeptides. Also provided are methods of using the polypeptides, for example, as an antitumor agent, and antiviral agent, and antibodies and antagonists against such polypeptides for example, for treating Alzheimer's disease, Parkinson's disease, rheumatoid arthritis and head injury. Diagnostic assays for identifying mutations in nucleic acid sequence encoding a polypeptide of the present invention and for detecting altered levels of the polypeptide of the present invention for detecting diseases are also disclosed.

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```
1          GCACGAGAAACTTTGCTGTGCGCGTTCTCCCGCGCGCGGGCTCAACTTTGTAGAGCGAGG

61         GGCCAACTTGGCAGAGCGCGCGGCCAGCTTTGCAGAGAGCGCCCTCCAGGGACTATGCGT
1                                                                M  R
121        GCGGGGACACGGGTCGCTTTGGGCTCTTCCACCCCTGCGGAGCGCACTACCCCGAGCCAG
3          A  G  T  R  V  A  L  G  S  S  T  P  A  E  R  T  T  P  S  Q
181        GGGCGGTGCAAGCCCCGCCCGGCCCTACCCAGGGCGGCTCCTCCCTCCGCAGCGCCGAGA
23         G  R  C  K  P  R  P  A  L  P  R  A  A  P  P  S  A  A  P  R
241        CTTTTAGTTTCGCTTTCGCTAAAGGGGCCCCAGACCCTTGCTGCGGAGCGACGGAGAGAG
43         L  L  V  S  L  S  L  K  G  P  Q  T  L  A  A  E  R  R  R  E
301        ACTGTGCCAGTCCCAGCCGCCCTACCGCCGTGGGAACGATGGCAGATGATTCAGGGCTGT
63         T  V  P  V  P  A  A  L  P  P  W  E  R  W  Q  M  I  Q  G  C
361        ATTGAAGAGCAGGGGGTTGAGGATTCAGCAAATGAAGATTCAGTGGATGCTAAGCCAGAC
83         I  E  E  Q  G  V  E  D  S  A  N  E  D  S  V  D  A  K  P  D
421        CGGTCCTCGTTTGTACCGTCCCTCTTCAGTAAGAAGAAGAAAAATGTCACCATGCGATCC
103        R  S  S  F  V  P  S  L  F  S  K  K  K  K  N  V  T  M  R  S
481        ATCAAGACCACCCGGGACCGAGTGCCTACATATCAGTACAACATGAATTTTGAAAAGCTG
123        I  K  T  T  R  D  R  V  P  T  Y  Q  Y  N  M  N  F  E  K  L
541        GGCAAATGCATCATAATAAACAACAAGAACTTTGATAAAGTGACAGGTATGGGCGTTCGA
143        G  K  C  I  I  I  N  N  K  N  F  D  K  V  T  G  M  G  V  R
601        AACGGAACAGACAAAGATGCCGAGGCGCTCTTCAAGTGCTTCCGAAGCCTGGGTTTTGAC
163        N  G  T  D  K  D  A  E  A  L  F  K  C  F  R  S  L  G  F  D
661        GTGATTGTCTATAATGACTGCTCTTGTGCCAAGATGCAAGATCTGCTTAAAAAAGCTTCT
183        V  I  V  Y  N  D  C  S  C  A  K  M  Q  D  L  L  K  K  A  S
721        GAAGAGGACCATACAAATGCCGCCTGCTTCGCCTGCATCCTCTTAAGCCATGGAGAAGAA
203        E  E  D  H  T  N  A  A  C  F  A  C  I  L  L  S  H  G  E  E
781        AATGTAATTTATGGGAAAGATGGTGTCACACCAATAAAGGATTTGACAGCCCACTTTAGG
223        N  V  I  Y  G  K  D  G  V  T  P  I  K  D  L  T  A  H  F  R
841        GGGGATAGATGCAAAACCCTTTTAGAGAAACCCAAACTCTTCTTCATTCAGGCTTGCCGA
243        G  D  R  C  K  T  L  L  E  K  P  K  L  F  F  I  Q  A  C  R
901        GGGACCGAGCTTGATGATGGCATCCAGGCCGACTCGGGGCCCATCAATGACACAGATGCT
263        G  T  E  L  D  D  G  I  Q  A  D  S  G  P  I  N  D  T  D  A
961        AATCCTCGATACAAGATCCCAGTGGAAGCTGACTTCCTCTTCGCCTATTCCACGGTTCCA
283        N  P  R  Y  K  I  P  V  E  A  D  F  L  F  A  Y  S  T  V  P
1021       GGCTATTACTCGTGGAGGAGCCCAGGAAGAGGCTCCTGGTTTGTGCAAGCCCTCTGCTCC
303        G  Y  Y  S  W  R  S  P  G  R  G  S  W  F  V  Q  A  L  C  S
1081       ATCCTGGAGGAGCACGGAAAAGACCTGGAAATCATGCAAATCCTCCACCAGGGTGAATGA
323        I  L  E  E  H  G  K  D  L  E  I  M  Q  I  L  H  Q  G  E  *
1141       CAGAGTTGCCAGGCACTTTGAGTCTCAGTCTGATGACCCACACTTCCATGAGAAGAAGCA

1201       GATCCCCTGTGTGGTCTCCATGCTCACCAAGGAACTCTACTTCAGTCAATAGCCATATCA

1261       GGGGTACATTCTAGCTGAGAAGCAATGGGTCACTCATTAATGAATCACATTTTTTTATGC

1321       TCTTGAAATATTCAGAAATTCTCCAGGATTTTAATTTCAGGAAAATGTATT
```

Figure 1

32580 -- -1 1 of 3

```
1       GCACGAGCGGATGGGTGCTATTGTGAGGCGGTTGTAGAAGAGTTTCGTGAGTGCTCGCAG

61      CTCATACCTGTGGCTGTGTATCCGTGGCCACAGCTGGTTGGCGTCGCCTTGAAATCCCAG

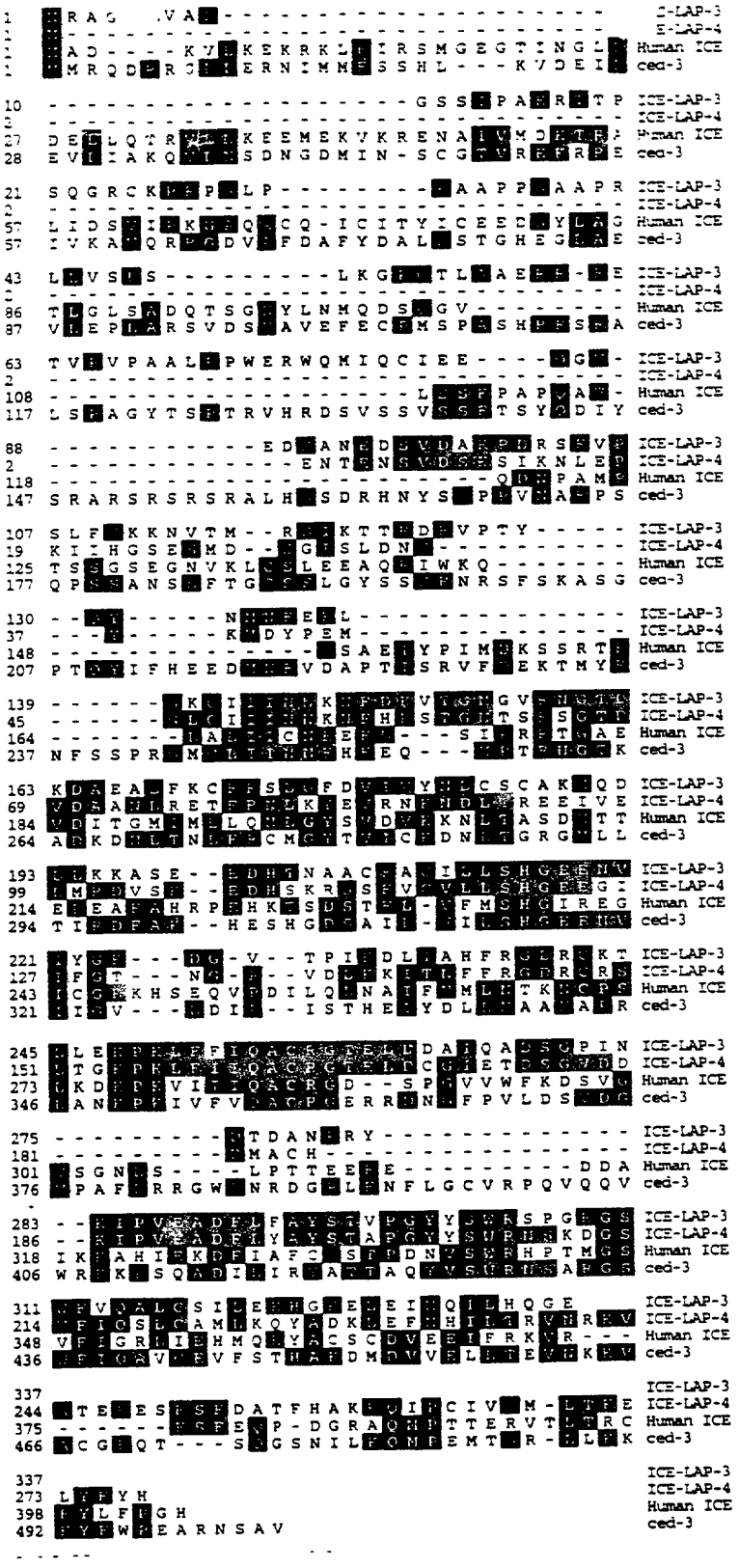
121     GCCGTGAGGAGTTAGCGAGCCCTGCTCACACTCGGCGCTCTGGTTTTCGGTGGGTGTGCC

181     CTGCACCTGCCTCTTCCCGCATTCTCATTAATAAAGGTATCCATGGAGAACACTGAAAAC
1                                                   M  E  N  T  E  N
241     TCAGTGGATTCAAAATCCATTAAAAATTTGGAACCAAAGATCATACATGGAAGCGAATCA
7       S  V  D  S  K  S  I  K  N  L  E  P  K  I  I  H  G  S  E  S
301     ATGGACTCTGGAATATCCCTGGACAACAGTTATAAAATGGATTATCCTGAGATGGGTTTA
27      M  D  S  G  I  S  L  D  N  S  Y  K  M  D  Y  P  E  M  G  L
361     TGTATAATAATTAATAATAAGAATTTTCATAAAAGCACTGGAATGACATCTCGGTCTGGT
47      C  I  I  I  N  N  K  N  F  H  K  S  T  G  M  T  S  R  S  G
421     ACAGATGTCGATGCAGCAAACCTCAGGGAAACATTCAGAAACTTGAAATATGAAGTCAGG
67      T  D  V  D  A  A  N  L  R  E  T  F  R  N  L  K  Y  E  V  R
481     AATAAAAATGATCTTACACGTGAAGAAATTGTGGAATTGATGCGTGATGTTTCTAAAGAA
87      N  K  N  D  L  T  R  E  E  I  V  E  L  M  R  D  V  S  K  E
541     GATCACAGCAAAAGGAGCAGTTTTGTTTGTGTGCTTCTGAGCCATGGTGAAGAAGGAATA
107     D  H  S  K  R  S  S  F  V  C  V  L  L  S  H  G  E  E  G  I
601     ATTTTTGGAACAAATGGACCTGTTGACCTGAAAAAAATAACAAACTTTTTCAGAGGGGAT
127     I  F  G  T  N  G  P  V  D  L  K  K  I  T  N  F  F  R  G  D
661     CGTTGTAGAAGTCTAACTGGAAAACCCAAACTTTTCATTATTCAGGCCTGCCGTGGTACA
147     R  C  R  S  L  T  G  K  P  K  L  F  I  I  Q  A  C  R  G  T
721     GAACTGGACTGTGGCATTGAGACAGACAGTGGTGTTGATGATGACATGGCGTGTCATAAA
167     E  L  D  C  G  I  E  T  D  S  G  V  D  D  D  M  A  C  H  K
781     ATACCAGTGGAGGCCGACTTCTTGTATGCATACTCCACAGCACCTGGTTATTATTCTTGG
187     I  P  V  E  A  D  F  L  Y  A  Y  S  T  A  P  G  Y  Y  S  W
841     CGAAATTCAAAGGATGGCTCCTGGTTCATCCAGTCGCTTTGTGCCATGCTGAAACAGTAT
207     R  N  S  K  D  G  S  W  F  I  Q  S  L  C  A  M  L  K  Q  Y
901     GCCGACAAGCTTGAATTTATGCACATTCTTACCCGGGTTAACCGAAAGGTGGCAACAGAA
227     A  D  K  L  E  F  M  H  I  L  T  R  V  N  R  K  V  A  T  E
961     TTTGAGTCCTTTTCCTTTGACGCTACTTTTCATGCAAAGAAACAGATTCCATGTATTGTT
247     F  E  S  F  S  F  D  A  T  F  H  A  K  K  Q  I  P  C  I  V
1021    TCCATGCTCACAAAAGAACTCTATTTTTATCACTAAAGAAATGGTTGGTTGGTGGTTTTT
267     S  M  L  T  K  E  L  Y  F  Y  H  *
1081    TTTAGTTTGTATGCCAAGTGAGAAGATGGTATATTTGGGTACTGTATTTCCCTCTCATTG

1141    GGGACCTACTCTCATGCTG
```

Figure 2

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Figure 3

Via Hand Delivery July 10, 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: He, et al.

Application Serial No.: Not yet assigned — Art Unit: Not yet assigned

Filed: Concurrently herewith — Examiner: Not yet assigned

For: Interleukin-1 ß Converting Enzyme Like Apoptosis Protease-3 and 4 — Attorney Docket No.: PF140P1D1

SUBMISSION OF FORMAL DRAWINGS

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Applicants submit herewith Formal Drawings of Figures 1A-B, 2A-B and 3A-C (7 sheets) to replace the informal drawings of Figures 1-3 (3 sheets).

No fee is believed due for this submission. In the event that a fee is required in connection with this submission, please charge the required fee to Deposit Account No. 08-3425.

Respectfully submitted,

Dated: July 10, 2000

Kenley K. Hoover (Reg. No. 40,302)
Attorney for Applicants

Human Genome Sciences, Inc.
9410 Key West Avenue
Rockville, MD 20850
(301) 251-6015 (phone)

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FIGURE 1A

```
   1  GCACGAGAAACTTTGCTGTGCGCGTTCTCCCGCGCGCGGGCTCAACTTTGTAGAGCGAGG   60

  61  GGCCAACTTGGCAGAGCGCGCGGCCAGCTTTGCAGAGAGCGCCCTCCAGGGACTATGCGT  120

 121  GCGGGGACACGGGTCGCTTTGGGCTCTTCCACCCCTGCGGAGCGCACTACCCCGAGCCAG  180

 181  GGGCGGTGCAAGCCCCGCCCGGCCCTACCCAGGGCGGCTCCTCCCTCCGCAGCGCCGAGA  240

 241  CTTTTAGTTTCGCTTTCGCTAAAGGGGCCCCAGACCCTTGCTGCGGAGCGACGGAGAGAG  300

 301  ACTGTGCCAGTCCCAGCCGCCCTACCGCCGTGGGAACGATGGCAGATGATCAGGGCTGTA  360
   1                                   M  A  D  D  Q  G  C  I    8

 361  TTGAAGAGCAGGGGGTTGAGGATTCAGCAAATGAAGATTCAGTGGATGCTAAGCCAGACC  420
   9   E  E  Q  G  V  E  D  S  A  N  E  D  S  V  D  A  K  P  D  R   28

 421  GGTCCTCGTTTGTACCGTCCCTCTTCAGTAAGAAGAAGAAAAATGTCACCATGCGATCCA  480
  29   S  S  F  V  P  S  L  F  S  K  K  K  K  N  V  T  M  R  S  I   48

 481  TCAAGACCACCCGGGACCGAGTGCCTACATATCAGTACAACATGAATTTTGAAAAGCTGG  540
  49   K  T  T  R  D  R  V  P  T  Y  Q  Y  N  M  N  F  E  K  L  G   68

 541  GCAAATGCATCATAATAAACAACAAGAACTTTGATAAAGTGACAGGTATGGGCGTTCGAA  600
  69   K  C  I  I  I  N  N  K  N  F  D  K  V  T  G  M  G  V  R  N   88

 601  ACGGAACAGACAAAGATGCCGAGGCGCTCTTCAAGTGCTTCCGAAGCCTGGGTTTTGACG  660
  89   G  T  D  K  D  A  E  A  L  F  K  C  F  R  S  L  G  F  D  V  108

 661  TGATTGTCTATAATGACTGCTCTTGTGCCAAGATGCAAGATCTGCTTAAAAAAGCTTCTG  720
 109   I  V  Y  N  D  C  S  C  A  K  M  Q  D  L  L  K  K  A  S  E  128

 721  AAGAGGACCATACAAATGCCGCCTGCTTCGCCTGCATCCTCTTAAGCCATGGAGAAGAAA  780
 129   E  D  H  T  N  A  A  C  F  A  C  I  L  L  S  H  G  E  E  N  148

 781  ATGTAATTTATGGGAAAGATGGTGTCACACCAATAAAGGATTTGACAGCCCACTTTAGGG  840
 149   V  I  Y  G  K  D  G  V  T  P  I  K  D  L  T  A  H  F  R  G  168

 841  GGGATAGATGCAAAACCCTTTTAGAGAAACCCAAACTCTTCTTCATTCAGGCTTGCCGAG  900
 169   D  R  C  K  T  L  L  E  K  P  K  L  F  F  I  Q  A  C  R  G  188

 901  GGACCGAGCTTGATGATGCCATCCAGGCCGACTCGGGGCCCATCAATGACACAGATGCTA  960
 189   T  E  L  D  D  A  I  Q  A  D  S  G  P  I  N  D  T  D  A  N  208
```

FIGURE 1B

```
961  ATCCTCGATACAAGATCCCAGTGGAAGCTGACTTCCTCTTCGCCTATTCCACGGTTCCAG  1020
209    P  R  Y  K  I  P  V  E  A  D  F  L  F  A  Y  S  T  V  P  G   228

1021 GCTATTACTCGTGGAGGAGCCCAGGAAGAGGCTCCTGGTTTGTGCAAGCCCTCTGCTCCA  1080
229    Y  Y  S  W  R  S  P  G  R  G  S  W  F  V  Q  A  L  C  S  I   248

1081 TCCTGGAGGAGCACGGAAAAGACCTGGAAATCATGCAGATCCTCACCAGGGTGAATGACA  1140
249    L  E  E  H  G  K  D  L  E  I  M  Q  I  L  T  R  V  N  D  R   268

1141 GAGTTGCCAGGCACTTTGAGTCTCAGTCTGATGACCCACACTTCCATGAGAAGAAGCAGA  1200
269    V  A  R  H  F  E  S  Q  S  D  D  P  H  F  H  E  K  K  Q  I   288

1201 TCCCCTGTGTGGTCTCCATGCTCACCAAGGAACTCTACTTCAGTCAATAGCCATATCAGG  1260
289    P  C  V  V  S  M  L  T  K  E  L  Y  F  S  Q                303

1261 GGTACATTCTAGCTGAGAAGCAATGGGTCACTCATTAATGAATCACATTTTTTTATGCTC  1320

1321 TTGAAATATTCAGAAATTCTCCAGGATTTTAATTTCAGGAAAATGTATT  1369
```

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FIGURE 2A

```
   1 GCACGAGCGGATGGGTGCTATTGTGAGGCGGTTGTAGAAGAGTTTCGTGAGTGCTCGCAG  60

  61 CTCATACCTGTGGCTGTGTATCCGTGGCCACAGCTGGTTGGCGTCGCCTTGAAATCCCAG 120

 121 GCCGTGAGGAGTTAGCGAGCCCTGCTCACACTCGGCGCTCTGGTTTTCGGTGGGTGTGCC 180

 181 CTGCACCTGCCTCTTCCCGCATTCTCATTAATAAAGGTATCCATGGAGAACACTGAAAAC 240
   1                                                M  E  N  T  E  N   6

 241 TCAGTGGATTCAAAATCCATTAAAAATTTGGAACCAAAGATCATACATGGAAGCGAATCA 300
   7  S  V  D  S  K  S  I  K  N  L  E  P  K  I  I  H  G  S  E  S  26

 301 ATGGACTCTGGAATATCCCTGGACAACAGTTATAAAATGGATTATCCTGAGATGGGTTTA 360
  27  M  D  S  G  I  S  L  D  N  S  Y  K  M  D  Y  P  E  M  G  L  46

 361 TGTATAATAATTAATAATAAGAATTTTCATAAAAGCACTGGAATGACATCTCGGTCTGGT 420
  47  C  I  I  I  N  N  K  N  F  H  K  S  T  G  M  T  S  R  S  G  66

 421 ACAGATGTCGATGCAGCAAACCTCAGGGAAACATTCAGAAACTTGAAATATGAAGTCAGG 480
  67  T  D  V  D  A  A  N  L  R  E  T  F  R  N  L  K  Y  E  V  R  86

 481 AATAAAAATGATCTTACACGTGAAGAAATTGTGGAATTGATGCGTGATGTTTCTAAAGAA 540
  87  N  K  N  D  L  T  R  E  E  I  V  E  L  M  R  D  V  S  K  E  106

 541 GATCACAGCAAAAGGAGCAGTTTTGTTTGTGTGCTTCTGAGCCATGGTGAAGAAGGAATA 600
 107  D  H  S  K  R  S  S  F  V  C  V  L  L  S  H  G  E  E  G  I  126

 601 ATTTTTGGAACAAATGGACCTGTTGACCTGAAAAAAATAACAAACTTTTTCAGAGGGGAT 660
 127  I  F  G  T  N  G  P  V  D  L  K  K  I  T  N  F  F  R  G  D  146

 661 CGTTGTAGAAGTCTAACTGGAAAACCCAAACTTTTCATTATTCAGGCCTGCCGTGGTACA 720
 147  R  C  R  S  L  T  G  K  P  K  L  F  I  I  Q  A  C  R  G  T  166

 721 GAACTGGACTGTGGCATTGAGACAGACAGTGGTGTTGATGATGACATGGCGTGTCATAAA 780
 167  E  L  D  C  G  I  E  T  D  S  G  V  D  D  D  M  A  C  H  K  186

 781 ATACCAGTGGAGGCCGACTTCTTGTATGCATACTCCACAGCACCTGGTTATTATTCTTGG 840
 187  I  P  V  E  A  D  F  L  Y  A  Y  S  T  A  P  G  Y  Y  S  W  206
```

FIGURE 2B

```
841 CGAAATTCAAAGGATGGCTCCTGGTTCATCCAGTCGCTTTGTGCCATGCTGAAACAGTAT 900
207  R  N  S  K  D  G  S  W  F  I  Q  S  L  C  A  M  L  K  Q  Y  226

901 GCCGACAAGCTTGAATTTATGCACATTCTTACCCGGGTTAACCGAAAGGTGGCAACAGAA 960
227  A  D  K  L  E  F  M  H  I  L  T  R  V  N  R  K  V  A  T  E  246

961 TTTGAGTCCTTTTCCTTTGACGCTACTTTTCATGCAAAGAAACAGATTCCATGTATTGTT 1020
247  F  E  S  F  S  F  D  A  T  F  H  A  K  K  Q  I  P  C  I  V  266

1021 TCCATGCTCACAAAAGAACTCTATTTTTATCACTAAAGAAATGGTTGGTTGGTGGTTTTT 1080
267  S  M  L  T  K  E  L  Y  F  Y  H  *                          277

1081 TTTAGTTTGTATGCCAAGTGAGAAGATGGTATATTTGGGTACTGTATTTCCCTCTCATTG 1140

1141 GGGACCTACTCTCATGCTG 1159
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0007/29 30SEP96

FIGURE 3A

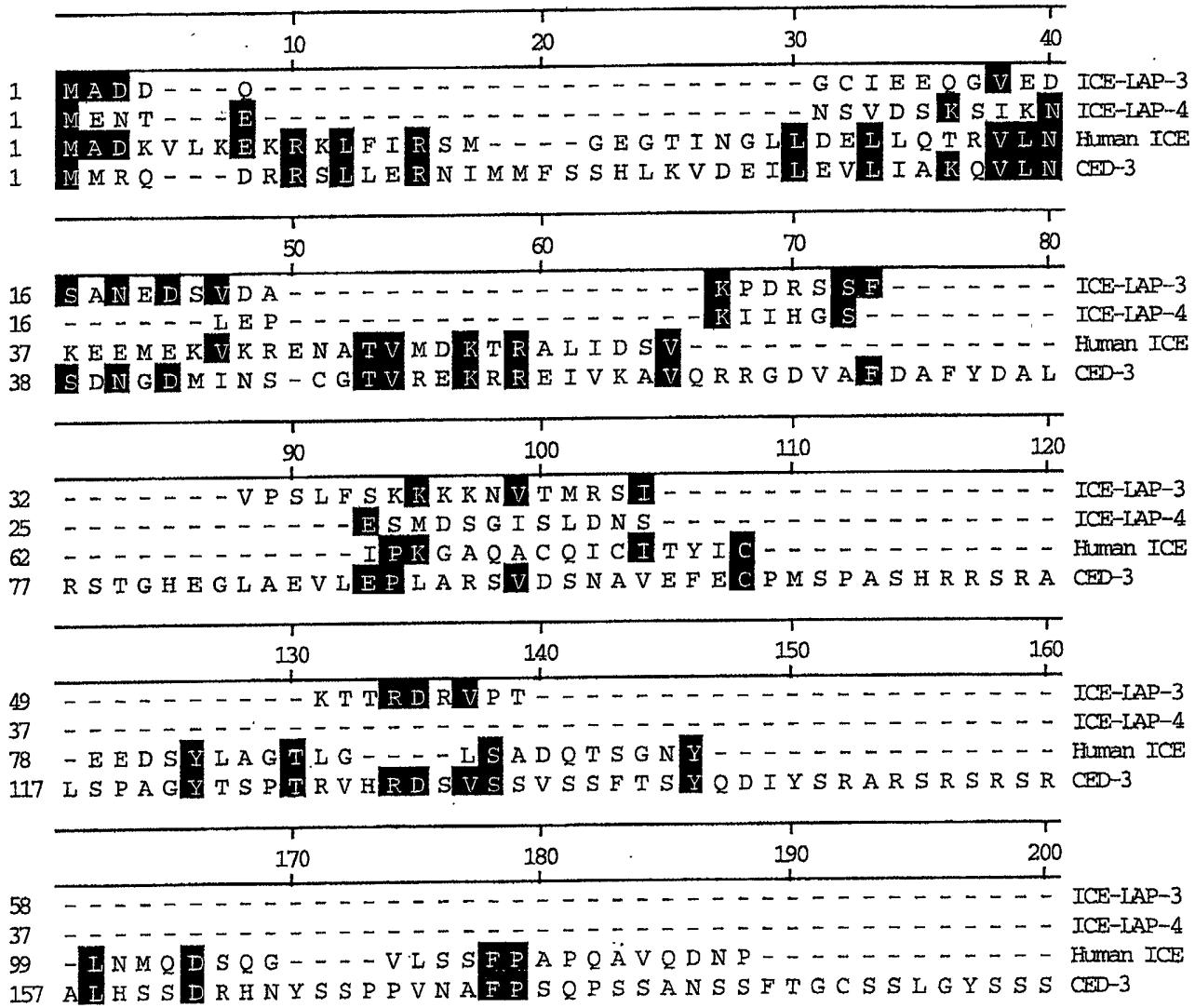


FIGURE 3B

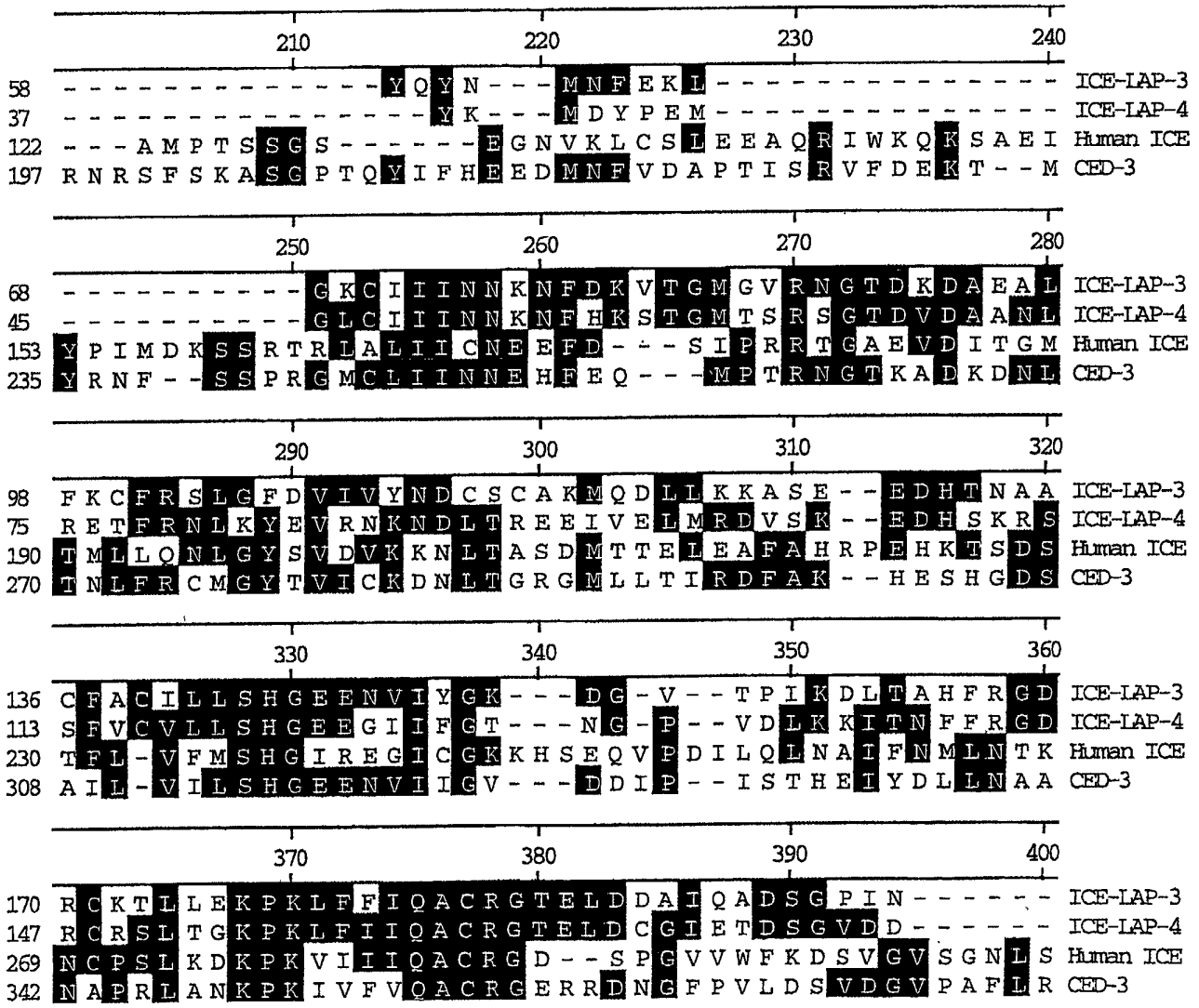
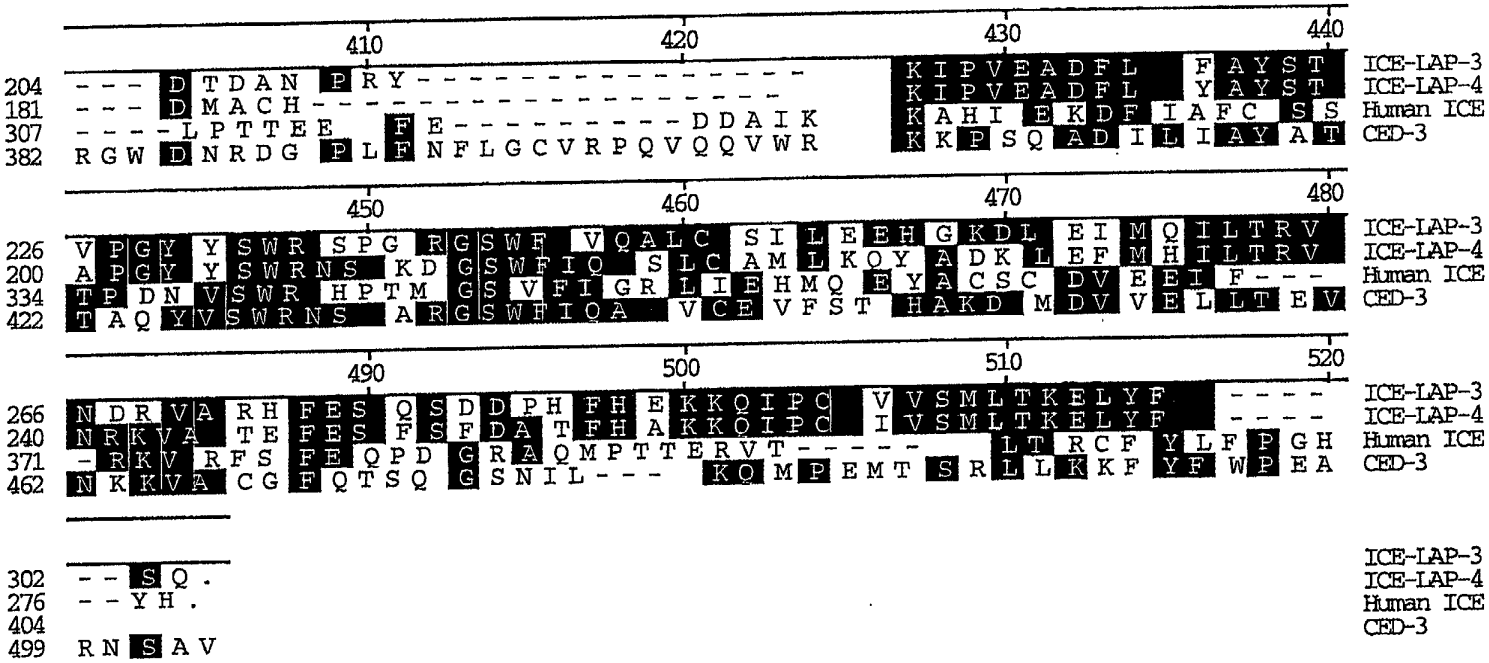

210 220 230 240
58 - - - - - - - - - - - - - - - Y Q Y N - - - M N F E K L - - - - - - - - - - - - - - - ICE-LAP-3
37 - - - - - - - - - - - - - - - Y K - - - M D Y P E M - - - - - - - - - - - - - - - ICE-LAP-4
122 - - - A M P T S S G S - - - - - - - E G N V K L C S L E E A Q R I W K Q K S A E I Human ICE
197 R N R S F S K A S G P T Q Y I F H E E D M N F V D A P T I S R V F D E K T - - M CED-3
250 260 270 280
68 - - - - - - - - - - - - G K C I I I N N K N F D K V T G M G V R N G T D K D A E A L ICE-LAP-3
45 - - - - - - - - - - - - G L C I I I N N K N F H K S T G M T S R S G T D V D A A N L ICE-LAP-4
153 Y P I M D K S S R T R L A L I I C N E E F D - - - S I P R R T G A E V D I T G M Human ICE
235 Y R N F - - S S P R G M C L I I N N E H F E Q - - - M P T R N G T K A D K D N L CED-3
290 300 310 320
98 F K C F R S L G F D V I V Y N D C S C A K M Q D L L K K A S E - - E D H T N A A ICE-LAP-3
75 R E T F R N L K Y E V R N K N D L T R E E I V E L M R D V S K - - E D H S K R S ICE-LAP-4
190 T M L L Q N L G Y S V D V K K N L T A S D M T T E L E A F A H R P E H K T S D S Human ICE
270 T N L F R C M G Y T V I C K D N L T G R G M L L T I R D F A K - - H E S H G D S CED-3
330 340 350 360
136 C F A C I L L S H G E E N V I Y G K - - - D G - V - - T P I K D L T A H F R G D ICE-LAP-3
113 S F V C V L L S H G E E G I I F G T - - - N G - P - - V D L K K I T N F F R G D ICE-LAP-4
230 T F L - V F M S H G I R E G I C G K K H S E Q V P D I L Q L N A I F N M L N T K Human ICE
308 A I L - V I L S H G E E N V I I G V - - - D D I P - - I S T H E I Y D L L N A A CED-3
370 380 390 400
170 R C K T L L E K P K L F F I Q A C R G T E L D D A I Q A D S G P I N - - - - - - ICE-LAP-3
147 R C R S L T G K P K L F I I Q A C R G T E L D C G I E T D S G V D D - - - - - - ICE-LAP-4
269 N C P S L K D K P K V I I I Q A C R G D - - S P G V V W F K D S V G V S G N L S Human ICE
342 N A P R L A N K P K I V F V Q A C R G E R R D N G F P V L D S V D G V P A F L R CED-3

FIGURE 3C



Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

Serial No 08/462,969
Ref No: PF140P1

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I declare that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Interleukin-1 β Converting Enzyme Like Apoptosis Protease 3 and 4

the specification of which was filed on June 5, 1995. Application Serial No: **08/462,969**

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application, which designated at least one country other than the United States listed below , and have also identified below any foreign application for patent or inventor's certificate , or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s):

			Priority Claimed Yes	No
(Number)	(Country)	(Day/Month/Year Filed)		

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or under § 365(b) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56 that became available between the filing date of the prior application and the national or PCT international filing date of this application.

08/334,251	**Nov-01-94**	**Pending**
(Application Serial No.)	(Filing Date)	(Status: patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: A. Anders Brookes (Reg. No. 36,373), James H. Davis (Reg. No. 40,582), Kenley Hoover (Reg. No. 40,302), Michele M. Wales (Reg. No. 43,975), and Joseph J. Kenny (Reg. No. 43,710),. of Human Genome Sciences, Inc. 9410 Key West Avenue, Rockville, Maryland, 20878.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first joint inventor: Wei-Wu He

Inventor's signature: _____ Date: 9/17/99
Residence: 6225 Free Stone Court Columbia, MD 21045 Citizenship: CN
Post Office Address: same as above

Full name of first joint inventor: Craig A. Rosen

Inventor's signature: _____ Date: _____
Residence: 22400 Rolling Hill Road Laytonsville, MD 20882 Citizenship: US
Post Office Address: same as above

Full name of additional joint inventor: Peter L. Hudson

Inventor's signature: _____ Date: _____
Residence: 19307 Penrod Terrace Germantown, MD 20874. Citizenship: US
Post Office Address: same as above

Full name of additional joint inventor: Gregg A. Hastings

Inventor's signature: _____ Date: _____
Residence: 31919 Richgrove Court Westlake Village, CA 91321 Citizenship: US
Post Office Address: same as above

Human Genome Sciences, Inc.
9410 Key West Avenue
Rockville, MD 20859

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I declare that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Interleukin-1 β Converting Enzyme Like Apoptosis Protease 3 and 4

the specification of which was filed on **June 5, 1995**. Application Serial No: **08/462,969**

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT international application, which designated at least one country other than the United States listed below , and have also identified below any foreign application for patent or inventor's certificate , or PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s):

			Priority Claimed	
			Yes	No
(Number)	(Country)	(Day/Month/Year Filed)		

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(Application Serial No.)	(Filing Date)

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08/334,251	**Nov-01-94**	**Pending**
(Application Serial No.)	(Filing Date)	(Status: patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: A. Anders Brookes (Reg. No. 36,373), James H. Davis (Reg. No. 40,582), Kenley Hoover (Reg. No. 40,302), Michele M. Wales (Reg. No. 43,975), and Joseph J. Kenny (Reg. No. 43,710), of Human Genome Sciences, Inc. 9410 Key West Avenue, Rockville, Maryland, 20878.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first joint inventor: Wei-Wu He

Inventor's signature: _____ Date: _____
Residence: 6225 Free Stone Court Columbia, MD 21045 Citizenship: CN
Post Office Address: same as above

Full name of first joint inventor: Craig A. Rosen

Inventor's signature: _____ Date: 4/14/97
Residence: 22400 Rolling Hill Road Laytonsville, MD 20882 Citizenship: US
Post Office Address: same as above

Full name of additional joint inventor: Peter L. Hudson

Inventor's signature: _____ Date: _____
Residence: 19307 Penrod Terrace Germantown, MD 20874 Citizenship: US
Post Office Address: same as above

Full name of additional joint inventor: Gregg A. Hastings

Inventor's signature: _____ Date: _____
Residence: 31919 Richgrove Court Westlake Village, CA 91321 Citizenship: US
Post Office Address: same as above

Human Genome Sciences, Inc.
9410 Key West Avenue
Rockville, MD 20859

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I declare that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

Interleukin-1 β Converting Enzyme Like Apoptosis Protease 3 and 4

the specification of which was filed on **June 5, 1995**. Application Serial No: **08/462,969**

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Prior Foreign Application(s):

			Priority Claimed	
(Number)	(Country)	(Day/Month/Year Filed)	Yes	No

I hereby claim the benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below.

(Application Serial No.)	(Filing Date)

I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or under § 365(b) of any PCT international application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information that is material to patentability as defined in 37 C.F.R. § 1.56 that became available between the filing date of the prior application and the national or PCT international filing date of this application.

08/334,251	**Nov-01-94**	**Pending**
(Application Serial No.)	(Filing Date)	(Status: patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: A. Anders Brookes (Reg. No. 36,373), James H. Davis (Reg. No. 40,582), Kenley Hoover (Reg. No. 40,302), Michele M. Wales (Reg. No. 43,975), and Joseph J. Kenny (Reg. No. 43,710), of Human Genome Sciences, Inc. 9410 Key West Avenue, Rockville, Maryland, 20878.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of first joint inventor: **Wei-Wu He**

Inventor's signature: _____ Date: _____
Residence: 6225 Free Stone Court Columbia, MD 21045 Citizenship: CN
Post Office Address: same as above

Full name of first joint inventor: **Craig A. Rosen**

Inventor's signature: _____ Date: _____
Residence: 22400 Rolling Hill Road Laytonsville, MD 20882 Citizenship: US
Post Office Address: same as above

Full name of additional joint inventor: **Peter L. Hudson**

Inventor's signature: _____ Date: 7/30/99
Residence: 19307 Penrod Terrace Germantown, MD 20874 Citizenship: US
Post Office Address: same as above

Full name of additional joint inventor: **Gregg A. Hastings**

Inventor's signature: _____ Date: 4/19/99
Residence: 31919 Richgrove Court Westlake Village, CA 91321 Citizenship: US
Post Office Address: same as above

Human Genome Sciences, Inc.
9410 Key West Avenue
Rockville, MD 20859

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: application of: He et al.

Serial No.: 08/462,969 Group Art Unit: 1652

Filed: June 5, 1995 Examiner: G. Bugaisky

For: Interleukin-1 β Converting Enzyme Like Apoptosis Protease 3 and 4 Attorney Docket No.: **PF140P1**

Declaration of Craig Rosen Under 37 CFR §1.132

Assistant Commissioner For Patents
Washington, D.C. 20231

Sir:

1. I, Craig Rosen, Ph.D., hereby declare and state as follows:

2. I am named as an inventor on the captioned U.S. Patent Application. I am employed by Human Genome Sciences, Inc. (HGS) as Senior Vice President, Research and Development. The work described below was done by myself, under my supervision, or as part of a collaborative research effort with other individuals at Human Genome Sciences, Inc. (HGS) and with individuals working at the University of Michigan.

3. We identified a cDNA clone encoding Interleukin-1 Beta Converting Enzyme Like Apoptosis Protease-3 (ICE-LAP3) by screening the proprietary HGS database of partial-length (EST) sequences for similarity to members of the ICE/Ced-3 polypeptide family. We retrieved the cDNA clone corresponding to the partial-length sequence from HGS' catalogued library of cDNA clones. The clone retrieved had the designation HE2CA82 and was deposited with the American Type Culture Collection as ATCC accession no. 75875 on August 25, 1994 (a copy of the ATCC contract for accession no. 75875 is attached as Exhibit A). We determined the complete nucleotide sequence for the HE2CA82 clone, using sequencing methods which were routine in the art and publicly available as of November 1, 1994; i.e., as of the filing date of parent application U.S. Serial No. 08/334,251, to which the captioned application claims priority.

4. The nucleotide sequence data filed in the captioned U.S. Patent Application and the nucleotide sequence data published in Duan et al., J. Biol. Chem. 271:1621 (1996) (Exhibit B), which differ slightly, were both obtained from cDNA clone HE2CA82. The "original" sequence data was disclosed in the captioned application and in parent application U.S. Serial No. 08/334,251. The "corrected" sequence data published in Duan et al. was also published in GenBank as accession no. U39613 (Exhibit C).

5. The differences in the original nucleotide sequence data and the corrected nucleotide sequence data are shown in the sequence alignment attached hereto as Exhibit D (the original sequence data is denoted "PF140" and the corrected sequence data is denoted "Duan"). As can be seen from Exhibit D, the original nucleotide sequence data differs from the corrected sequence data at four (4) nucleotide positions. The differences in the originally deduced amino acid sequence and the amino acid sequence deduced from the corrected nucleotide sequence data is shown in the alignment attached hereto as Exhibit E.

6. The accuracy of the original sequencing data represented by the sequences shown in Figure 1 of the present application is greater than 99%. I am of the opinion that 99% accuracy is well within the state of the art of DNA sequencing at the time parent application U.S. Serial No. 08/334,251 was filed (i.e., November 1, 1994). Further, I am of the opinion that the corrected ICE-LAP3 nucleotide and amino acid sequences would have been apparent to one skilled in the art in possession of the corresponding human cDNA clone contained in ATCC Deposit No. 75875 (referenced in the captioned patent application and in parent application U.S. Serial No. 08/334,251) and the sequence data presented in U.S. Serial No. 08/334,251. I believe this to be true because the correct ICE-LAP3 coding sequence can be readily determined from the deposited clone using methods of DNA sequencing which were routine in the art as of November 1, 1994.

7. I believe that the actual coding portion of the nucleotide sequence of the human cDNA clone contained in ATCC Deposit No. 75875 is the same as the nucleotide sequence published as GenBank Accession No. U39613 in connection with the Duan et al. publication.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereupon.

3/14/99
Date

Craig Rosen, Ph.D.

Via Hand Delivery July 10, 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: He, et al.

Application No.: Not yet assigned — Art Unit: Not yet assigned

Filed: Concurrently herewith — Examiner: Not yet assigned

For: Interleukin-1 ß Converting Enzyme Like Apoptosis Protease-3 and 4 — Attorney Docket No.: PF140P1D1

POWER OF ATTORNEY BY ASSIGNEE OF ENTIRE INTEREST
Revocation of Prior Power of Attorney

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Human Genome Sciences, Inc., organized and existing under the laws of the State of Delaware, having its principal place of business at 9410 Key West Avenue, Rockville, Maryland 20850, is assignee of record of the entire right, title and interest for the above-identified application by virtue of an assignment filed in the parent application U.S. Application Serial No. 08/462,969, filed June 5, 1995, and recorded on April 22, 1996 in the U.S. Patent and Trademark Office at Reel 7937, Frame 0822.

The undersigned has reviewed all the documents in the chain of title of the patent application identified above and, to the best of undersigned's knowledge and belief, title is in the assignee identified above.

The assignee hereby revokes all powers of attorney heretofore given in the above-captioned application and appoints as its attorneys or agents James H. Davis, (Registration No. 40,582); Kenley K. Hoover, (Registration No. 40,302); Joseph Kenny (Registration No. 43,710); Jonathan L. Klein (Registration No. 41, 119); and Michele Wales (Registration No. 43,975) with full power of substitution, association, and revocation, to prosecute said application and to transact all business in the United States Patent and Trademark Office connected therewith.

Please retain the correspondence address for this application as:

Human Genome Sciences, Inc.
9410 Key West Avenue
Rockville, Maryland 20850
Tel: 301-309-8504 Fax: 301-309-8439

The undersigned, whose title is supplied below, is empowered to sign this document on behalf of the assignee.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under Section 1001, Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application captioned above or of any patent issuing thereon.

On behalf of Human Genome Sciences, Inc.

For: Human Genome Sciences, Inc

Signature: J H D

Name: James H. Davis, Ph.D.

Title: Senior Vice President and General Counsel

Date: July 10, 2000

09654900 - 071900

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: He, Wei-Wu et al.

(ii) TITLE OF INVENTION: Interleukin-1 Beta Converting Enzyme Like Apoptosis Protease 3 and 4

(iii) NUMBER OF SEQUENCES: 12

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Human Genome Sciences, Inc.
(B) STREET: 9410 Key West Ave.
(C) CITY: Rockville
(D) STATE: MD
(E) COUNTRY: USA
(F) ZIP: 20850

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/462,969
(B) FILING DATE: 05-JUN-1995
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 08/334,251
(B) FILING DATE: 11-NOV-1994

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Brookes, A. Anders
(B) REGISTRATION NUMBER: 36,373
(C) REFERENCE/DOCKET NUMBER: PF140P1

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 301-309-8504
(B) TELEFAX: 301-309-8439

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1371 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GCACGAGAAA CTTTGCTGTG CGCGTTCTCC CGCGCGCGGG CTCAACTTTG TAGAGCGAGG 60

GGCCAACTTG GCAGAGCGCG CGGCCAGCTT TGCAGAGAGC GCCCTCCAGG GACTATGCGT 120

GCGGGGACAC GGGTCGCTTT GGGCTCTTCC ACCCCTGCGG AGCGCACTAC CCCGAGCCAG 180

GGGCGGTGCA AGCCCCGCCC GGCCCTACCC AGGGCGGCTC CTCCCTCCGC AGCGCCGAGA 240

CTTTTAGTTT CGCTTTCGCT AAAGGGGCCC CAGACCCTTG CTGCGGAGCG ACGGAGAGAG 300

ACTGTGCCAG TCCCAGCCGC CCTACCGCCG TGGGAACGAT GGCAGATGAT TCAGGGCTGT 360

ATTGAAGAGC AGGGGGTTGA GGATTCAGCA AATGAAGATT CAGTGGATGC TAAGCCAGAC 420

CGGTCCTCGT TTGTACCGTC CCTCTTCAGT AAGAAGAAGA AAAATGTCAC CATGCGATCC 480

ATCAAGACCA CCCGGGACCG AGTGCCTACA TATCAGTACA ACATGAATTT TGAAAAGCTG 540

GGCAAATGCA TCATAATAAA CAACAAGAAC TTTGATAAAG TGACAGGTAT GGGCGTTCGA 600

AACGGAACAG ACAAAGATGC CGAGGCGCTC TTCAAGTGCT TCCGAAGCCT GGGTTTTGAC 660

GTGATTGTCT ATAATGACTG CTCTTGTGCC AAGATGCAAG ATCTGCTTAA AAAAGCTTCT 720

GAAGAGGACC ATACAAATGC CGCCTGCTTC GCCTGCATCC TCTTAAGCCA TGGAGAAGAA 780

AATGTAATTT ATGGGAAAGA TGGTGTCACA CCAATAAAGG ATTTGACAGC CCACTTTAGG 840

GGGGATAGAT GCAAAACCCT TTTAGAGAAA CCCAAACTCT TCTTCATTCA GGCTTGCCGA 900

GGGACCGAGC TTGATGATGG CATCCAGGCC GACTCGGGGC CCATCAATGA CACAGATGCT 960

AATCCTCGAT ACAAGATCCC AGTGGAAGCT GACTTCCTCT TCGCCTATTC CACGGTTCCA 1020

GGCTATTACT CGTGGAGGAG CCCAGGAAGA GGCTCCTGGT TTGTGCAAGC CCTCTGCTCC 1080

ATCCTGGAGG AGCACGGAAA AGACCTGGAA ATCATGCAAA TCCTCCACCA GGGTGAATGA 1140

CAGAGTTGCC AGGCACTTTG AGTCTCAGTC TGATGACCCA CACTTCCATG AGAAGAAGCA 1200

GATCCCCTGT GTGGTCTCCA TGCTCACCAA GGAACTCTAC TTCAGTCAAT AGCCATATCA 1260

GGGGTACATT CTAGCTGAGA AGCAATGGGT CACTCATTAA TGAATCACAT TTTTTTATGC 1320

TCTTGAAATA TTCAGAAATT CTCCAGGATT TTAATTTCAG GAAAATGTAT T 1371

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 303 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Met Ala Asp Asp Gln Gly Cys Ile Glu Glu Gln Gly Val Glu Asp Ser

1 5 10 15

Ala Asn Glu Asp Ser Val Asp Ala Lys Pro Asp Arg Ser Ser Phe Val
20 25 30

Pro Ser Leu Phe Ser Lys Lys Lys Lys Asn Val Thr Met Arg Ser Ile
35 40 45

Lys Thr Thr Arg Asp Arg Val Pro Thr Tyr Gln Tyr Asn Met Asn Phe
50 55 60

Glu Lys Leu Gly Lys Cys Ile Ile Ile Asn Asn Lys Asn Phe Asp Lys
65 70 75 80

Val Thr Gly Met Gly Val Arg Asn Gly Thr Asp Lys Asp Ala Glu Ala
85 90 95

Leu Phe Lys Cys Phe Arg Ser Leu Gly Phe Asp Val Ile Val Tyr Asn
100 105 110

Asp Cys Ser Cys Ala Lys Met Gln Asp Leu Leu Lys Lys Ala Ser Glu
115 120 125

Glu Asp His Thr Asn Ala Ala Cys Phe Ala Cys Ile Leu Leu Ser His
130 135 140

Gly Glu Glu Asn Val Ile Tyr Gly Lys Asp Gly Val Thr Pro Ile Lys
145 150 155 160

Asp Leu Thr Ala His Phe Arg Gly Asp Arg Cys Lys Thr Leu Leu Glu
165 170 175

Lys Pro Lys Leu Phe Phe Ile Gln Ala Cys Arg Gly Thr Glu Leu Asp
180 185 190

Asp Ala Ile Gln Ala Asp Ser Gly Pro Ile Asn Asp Thr Asp Ala Asn
195 200 205

Pro Arg Tyr Lys Ile Pro Val Glu Ala Asp Phe Leu Phe Ala Tyr Ser
210 215 220

Thr Val Pro Gly Tyr Tyr Ser Trp Arg Ser Pro Gly Arg Gly Ser Trp
225 230 235 240

Phe Val Gln Ala Leu Cys Ser Ile Leu Glu Glu His Gly Lys Glu Leu
245 250 255

Glu Ile Met Gln Ile Leu Thr Arg Val Asn Asp Arg Val Ala Arg His
260 265 270

Phe Glu Ser Gln Ser Asp Asp Pro His Phe His Glu Lys Lys Gln Ile
275 280 285

Pro Cys Val Val Ser Met Leu Thr Lys Glu Leu Tyr Phe Ser Gln
290 295 300

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1159 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GCACGAGCGG ATGGGTGCTA TTGTGAGGCG GTTGTAGAAG AGTTTCGTGA GTGCTCGCAG 60

CTCATACCTG TGGCTGTGTA TCCGTGGCCA CAGCTGGTTG GCGTCGCCTT GAAATCCCAG 120

GCCGTGAGGA GTTAGCGAGC CCTGCTCACA CTCGGCGCTC TGGTTTTCGG TGGGTGTGCC 180

CTGCACCTGC CTCTTCCCGC ATTCTCATTA ATAAAGGTAT CCATGGAGAA CACTGAAAAC 240

TCAGTGGATT CAAAATCCAT TAAAAATTTG GAACCAAAGA TCATACATGG AAGCGAATCA 300

ATGGACTCTG GAATATCCCT GGACAACAGT TATAAAATGG ATTATCCTGA GATGGGTTTA 360

TGTATAATAA TTAATAATAA GAATTTTCAT AAAAGCACTG GAATGACATC TCGGTCTGGT 420

ACAGATGTCG ATGCAGCAAA CCTCAGGGAA ACATTCAGAA ACTTGAAATA TGAAGTCAGG 480

AATAAAAATG ATCTTACACG TGAAGAAATT GTGGAATTGA TGCGTGATGT TTCTAAAGAA 540

GATCACAGCA AAAGGAGCAG TTTTGTTTGT GTGCTTCTGA GCCATGGTGA AGAAGGAATA 600

ATTTTTGGAA CAAATGGACC TGTTGACCTG AAAAAAATAA CAAACTTTTT CAGAGGGGAT 660

CGTTGTAGAA GTCTAACTGG AAAACCCAAA CTTTTCATTA TTCAGGCCTG CCGTGGTACA 720

GAACTGGACT GTGGCATTGA GACAGACAGT GGTGTTGATG ATGACATGGC GTGTCATAAA 780

ATACCAGTGG AGGCCGACTT CTTGTATGCA TACTCCACAG CACCTGGTTA TTATTCTTGG 840

CGAAATTCAA AGGATGGCTC CTGGTTCATC CAGTCGCTTT GTGCCATGCT GAAACAGTAT 900

GCCGACAAGC TTGAATTTAT GCACATTCTT ACCCGGGTTA ACCGAAAGGT GGCAACAGAA 960

TTTGAGTCCT TTTCCTTTGA CGCTACTTTT CATGCAAAGA AACAGATTCC ATGTATTGTT 1020

TCCATGCTCA CAAAAGAACT CTATTTTTAT CACTAAAGAA ATGGTTGGTT GGTGGTTTTT 1080

TTTAGTTTGT ATGCCAAGTG AGAAGATGGT ATATTTGGGT ACTGTATTTC CCTCTCATTG 1140

GGGACCTACT CTCATGCTG 1159

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 277 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Met Glu Asn Thr Glu Asn Ser Val Asp Ser Lys Ser Ile Lys Asn Leu
1 5 10 15

Glu Pro Lys Ile Ile His Gly Ser Glu Ser Met Asp Ser Gly Ile Ser
20 25 30

Leu Asp Asn Ser Tyr Lys Met Asp Tyr Pro Glu Met Gly Leu Cys Ile
35 40 45

Ile Ile Asn Asn Lys Asn Phe His Lys Ser Thr Gly Met Thr Ser Arg
50 55 60

Ser Gly Thr Asp Val Asp Ala Ala Asn Leu Arg Glu Thr Phe Arg Asn
65 70 75 80

Leu Lys Tyr Glu Val Arg Asn Lys Asn Asp Leu Thr Arg Glu Glu Ile
85 90 95

Val Glu Leu Met Arg Asp Val Ser Lys Glu Asp His Ser Lys Arg Ser
100 105 110

Ser Phe Val Cys Val Leu Leu Ser His Gly Glu Glu Gly Ile Ile Phe
115 120 125

Gly Thr Asn Gly Pro Val Asp Leu Lys Lys Ile Thr Asn Phe Phe Arg
130 135 140

Gly Asp Arg Cys Arg Ser Leu Thr Gly Lys Pro Lys Leu Phe Ile Ile
145 150 155 160

Gln Ala Cys Arg Gly Thr Glu Leu Asp Cys Gly Ile Glu Thr Asp Ser
165 170 175

Gly Val Asp Asp Asp Met Ala Cys His Lys Ile Pro Val Glu Ala Asp
180 185 190

Phe Leu Tyr Ala Tyr Ser Thr Ala Pro Gly Tyr Tyr Ser Trp Arg Asn
195 200 205

Ser Lys Asp Gly Ser Trp Phe Ile Gln Ser Leu Cys Ala Met Leu Lys
210 215 220

Gln Tyr Ala Asp Lys Leu Glu Phe Met His Ile Leu Thr Arg Val Asn
225 230 235 240

Arg Lys Val Ala Thr Glu Phe Glu Ser Phe Ser Phe Asp Ala Thr Phe
245 250 255

His Ala Lys Lys Gln Ile Pro Cys Ile Val Ser Met Leu Thr Lys Glu
260 265 270

Leu Tyr Phe Tyr His
275

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

GATCGGATCC ATGCGTGCGG GGACACGGGT C 31

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GTACTCTAGA TCATTCACCC TGGTGGAGGA T 31

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GATCGGATCC ATGGAGAACA CTGAAAACTC A 31

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

GTACTCTAGA TTAGTGATAA AAATAGAGTT C 31

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACTATGCGT GCGGGGACAC GG 22

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AATCAAGCGT AGTCTGGGAC GTCGTATGGG TATTCACCCT GGTGGAGGAT TTG 53

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

ACCATGGAGA ACACTGAAAA C 21

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 53 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AATCAAGCGT AGTCTGGGAC GTCGTATGGG TAGTGATAAA AATAGAGTTC TTT 53

SUBSTITUTE SHEET (RULE 26)